Studies on the Biosynthesis of Clavulanic Acid. Part 5.[1] Absolute Stereochemistry of Proclavaminic Acid, the Monocyclic Biosynthetic Precursor of Clavulanic Acid

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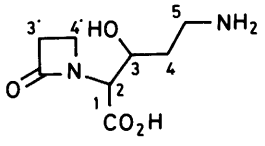
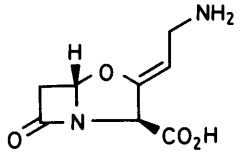
Proclavaminic acid (**1**) was synthesized by a route which indicated its constitution to be (2*S*,3*R*)-5-amino-3-hydroxy-2-(2-oxoazetidin-1-yl)valeric acid. The spectroscopic properties of the synthetic material were identical with those of natural proclavaminic acid, and, like the natural product, it was converted into clavaminic acid (**2**) by clavaminic acid synthase. An efficient synthesis of 3-hydroxyornithine derivatives was devised which allowed the separation of diastereoisomers and the resolution of a *threo* compound by the acylase [EC 3.5.1.11] from *Escherichia coli*. The β-lactam ring was subsequently elaborated by Michael addition of a protected 3-hydroxyornithine to acrylic acid followed by ring closure using triphenylphosphine/di-2-pyridyl disulphide. Model reactions were carried out with enantiomerically pure threonine derivatives to confirm that the formation of the β-lactam moiety did not impair the integrity of the α- and β-chiral centres and that the enzymatic deacylation reaction was capable of resolving the α-centre of an α-amino-β-hydroxy acid. The enantiomeric purity of intermediates was determined using HPLC, ^{1}H NMR spectroscopy utilising the chiral solvating reagents (*R*)- and (*S*)-1-(9-anthryl)-2,2,2-trifluoroethanol, and chiral GLC techniques.

Recent preliminary communications from these laboratories described the isolation of two novel intracellular β-lactams, proclavaminic acid (**1**) and clavaminic acid (**2**),[2] from *Streptomyces clavuligerus* and the roles of the two compounds in the biosynthesis of clavulanic acid (**3**) in this organism.[3] A synthesis of proclavaminic acid (**1**) which did not allow the stereochemistry to be assigned was described,[1,4] and more recently the elucidation of the absolute stereochemistry was reported.[5] In this paper a full description of the work leading to the discovery of the absolute stereochemistry of proclavaminic acid (**1**) is presented.



(**1**)



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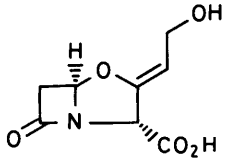
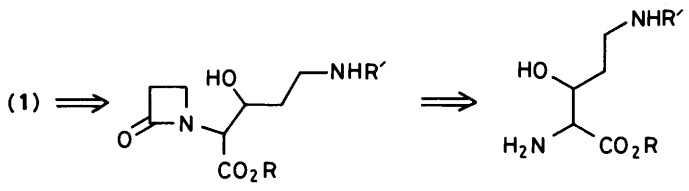


(**3**)

In order to assign the absolute stereochemistry to proclavaminic acid, synthetic routes were required which allowed the synthesis of all four possible stereoisomers of known absolute stereochemistry. The assignment would depend on one of these synthetic isomers being converted into compound (**2**) by clavaminic acid synthase.[2] 3-Hydroxyornithines of known relative stereochemistry have been reported[6] and methods for the resolution of amino acids are well documented,[7] hence the synthetic problem reduced to the elaboration of a β-lactam moiety onto the α-amino function of a protected 3-hydroxyornithine (**4**) and subsequent deprotection. The following factors were deemed essential for such a route to be successful. (i) The method of elaborating the β-lactam ring must not affect the chiral integrity of the resolved 3-hydroxyornithine. (ii) Protecting groups used during the synthesis of the 3-hydroxyornithine should allow separation of diastereoisomers and their resolution, and be compatible with subsequent β-lactam formation. (iii) A reference sample of a suitable 3-hydroxyornithine derivative of known relative stereochemistry would be required to identify diastereoisomers produced in the synthetic route. (iv) A method capable of resolving the α-centre of α-amino-β-hydroxy acid would be required.



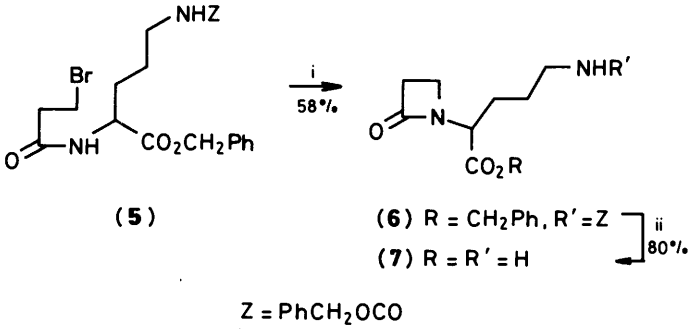
(**4**)

How these criteria were met, and the subsequent successful synthesis of biologically active, enantiomerically pure proclavaminic acid, are described below.

Results and Discussion

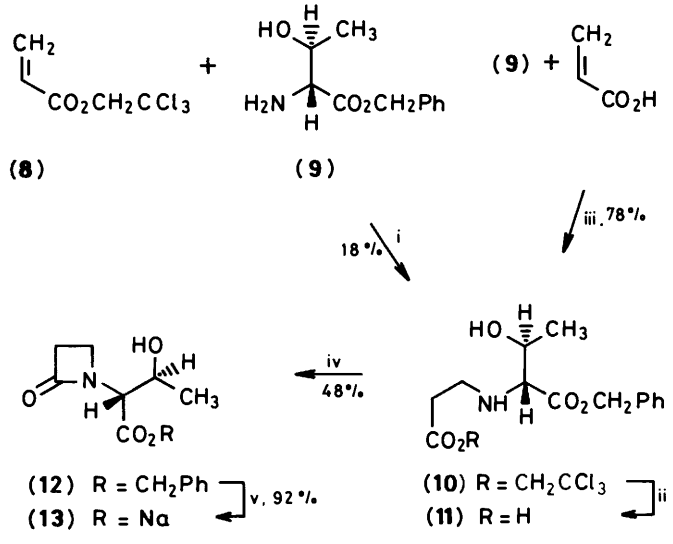
Elaboration of the Azetidinone Ring.—The construction of unsubstituted β-lactams onto the amino function of amino acid derivatives has been reported using oxidative ring expansion,[8]

and ring closures of β-amino acids[9,10] and β-substituted propionamides.[11,12] Our initial attempts to prepare dehydroxyproclavaminic acid (7) from a protected ornithine involved the base-catalysed cyclisation[12] of the two enantiomeric 3-bromopropionamides (5) followed by catalytic reduction of the protected amino acid (6) (Scheme 1). The azetidinones (7) from



Scheme 1. *Reagents and conditions:* i, $Bu_4N^+ Br^-$, KOH, dichloromethane (DCM)–MeCN (19:1). ii, H_2, Pd–C (10%), EtOAc–EtOH (7:3).

(*S*)- and (*R*)-(**6**) showed opposite and approximately equal rotations of −4.8° and +5.3° respectively. However, an examination of the resultant products by HPLC of an (*R*)-phenylalanine derivative revealed each material to be a mixture of enantiomers in the ratios 3:2 and 2:3 indicating that the base-cyclisation method caused considerable racemisation. The formation of β-lactams from β-amino acid derivatives using the Mukaiyama–Ohno conditions (triphenylphosphine–di-2-pyridyl disulphide in acetonitrile)[9] has proved successful in a number of varied situations.[13] Therefore this approach was investigated using (2*S*,3*R*)- and (2*R*,3*S*)-threonine benzyl esters as model compounds to determine whether this method could be utilised without affecting the chiral centres of α-amino-β-hydroxy esters. Condensation of 2,2,2-trichloroethyl acrylate (**8**)[14] with (2*R*,3*S*)-threonine benzyl ester (**9**)[15] in ethanol[16] yielded the expected[17] Michael addition product (**10**), which was selectively deprotected with zinc–acetic acid to afford the β-amino acid (**11**) in poor overall yield (Scheme 2). However, addition of acrylic acid (10 mol equiv.) to the ester (**9**) in acetonitrile to give acid (**11**) proceeded in 78% yield after a simple isolation procedure, thus avoiding a deprotection stage.

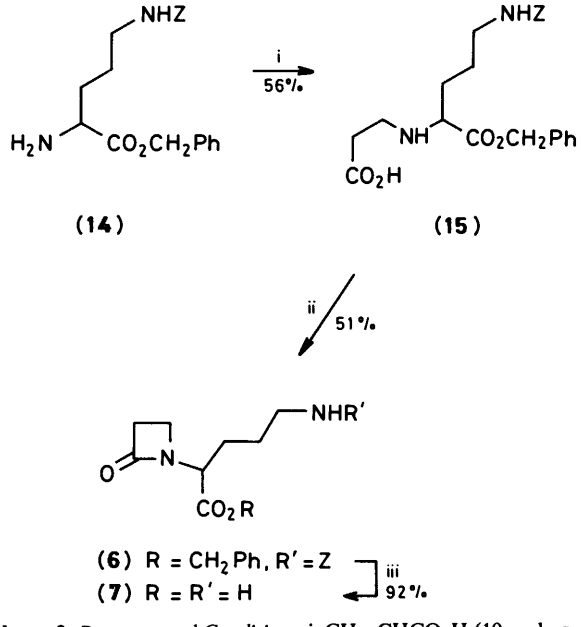


Scheme 2. *Reagents and conditions:* i, EtOH, room temp. ii, Zn–AcOH, THF. iii, MeCN, room temp. iv, di-2-pyridyl disulphide, Ph_3P, MeCN, reflux. v, H_2, Pd–C (10%), EtOH–water (2:1) and then NaOH.

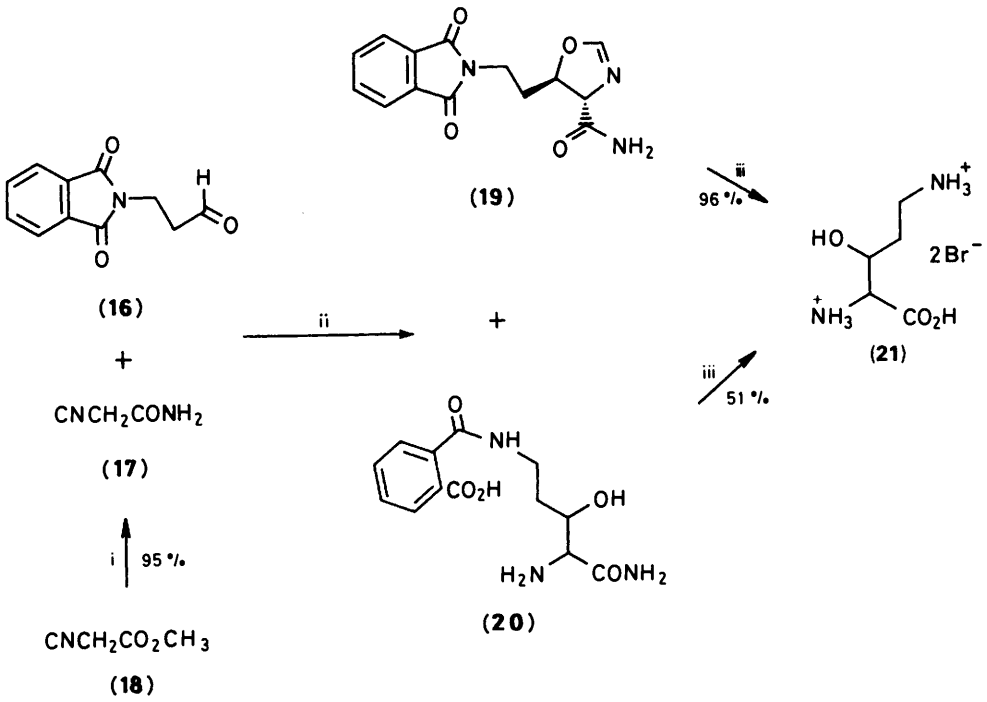
This general approach to the preparation of substituted β-amino acids for subsequent cyclisation to substituted β-lactams was described in 1958 by Blicke and Gould.[18] Ring closure of the acids (**11**) derived from (2*S*,3*R*)-(**9**) and (2*R*,3*S*)-(**9**) by the Ohno procedure yielded the respective enantiomers (**12**). The optical rotations of (2*S*,3*R*)-(**12**) and (2*R*,3*S*)-(**12**) were −4.60° and +4.55 respectively. The ^1H NMR spectrum of (2*S*,3*R*)-(**12**) in the presence of ten times its weight of the chiral solvating reagent (*R*)-1-(9-anthryl)-2,2,2-trifluoroethanol[19] resulted in no separation of signals. However when a mixture of enantiomers of compound (**12**) was run under the same conditions, upfield shifts, and separation (Δδ 0.038) of the doublets due to the non-equivalent protons at the 2-position of the two enantiomers, were observed. None of the other protons of compound (**12**) exhibited significant non-equivalence under these conditions. These experiments indicated that the formation of the β-lactam had occurred without affecting the chiral integrity of the 2- and 3-position. The corresponding sodium salts (**13**) were obtained by catalytic hydrogenation and gave rotations of −24.29° and +22.80°.

Treatment of (2*S*,3*R*)-(**12**) with 1,5-diazabicyclo[4.3.0]non-5-ene (DBN) in dichloromethane (DCM) followed by chromatography gave a 4:1 (*threo*:*erythro*) mixture of diastereoisomers of compound (**12**) (^1H NMR), confirming that epimerisation at the 2-position in the cyclisation reaction could be readily detected. This reaction also demonstrated the possibility for interconverting diastereoisomers in this system.

Since the 5-amino function of the 3-hydroxyornithine will need masking during elaboration of the β-lactam ring onto the 2-amino function, the stability of a carbamate group to the ring-elaboration method was tested on the benzyl ester of (*S*)-N^5-benzyloxycarbonylornithine, (**14**),[20] the most applicable model with respect to the envisaged synthesis. Condensation of acrylic acid and the protected ornithine (**14**) yielded the β-amino acid (**15**) (56%) which cyclised under the Ohno conditions to the azetidinone (**6**) (Scheme 3). The ^1H NMR spectrum of compound (**6**) obtained in the presence of (*R*)-1-(9-anthryl)-2,2,2-trifluoroethanol demonstrated this material to be enantiomerically pure. Catalytic reduction gave the dehydroxyproclavaminic acid (**7**) which was shown to be enantiomerically pure by HPLC.



Scheme 3. *Reagents and Conditions:* i, CH_2=$CHCO_2H$ (10 mol equiv.), MeCN, room temp. ii, di-2-pyridyl disulphide, Ph_3P, MeCN, reflux. iii, H_2, Pd–C (10%), EtOH–water (2:1).



Scheme 4. *Reagents and conditions:* i, NH_3, MeOH. ii, KOH (1 mol equiv.), MeOH, 0–5 °C. iii, 47% HBr, anisole, reflux.

The results described above indicated that the developed methods are suitable for construction of a β-lactam onto an enantiomerically pure benzyl ester of N^5-benzyloxycarbonyl-3-hydroxyornithine in a direct manner whilst maintaining the chiral integrity of both chiral centres. The observation of non-equivalence of the 2-protons in the ^1H NMR spectra of compounds (**6**) and (**12**) in the presence of the chiral solvating reagent was of considerable use in this study. No correlation of the absolute stereochemistry of the 2-proton of these compounds and the sense of the field shift was discernible. However, correlations have been reported for 2-amino esters.[21]

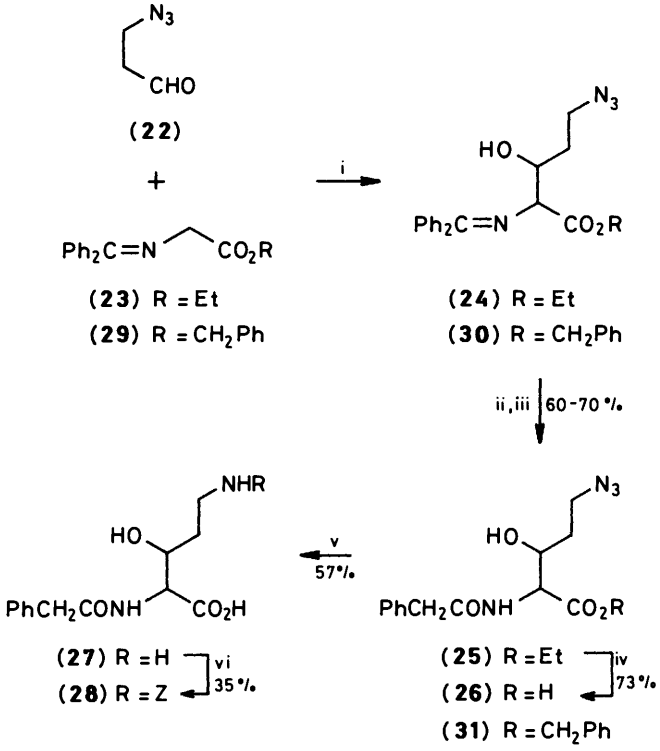
Strategy for Resolution of the α-Centre.—Since it seemed unlikely that the chirality of the 2-position of proclavaminic acid (**1**) would be affected in the enzymatic cyclisation to clavaminic acid, a synthetic strategy was developed to enable the (2*S*)-stereoisomers of N^5-protected 3-hydroxyornithine diastereoisomers to be prepared with the facility for the redirection of intermediates to the 2*R*-series should the need arise. The ready availability to us of immobilised *E. coli* acylase [EC 3.5.1.11],[22] which is known selectively to deacylate N^2-phenylacetyl (2*S*)-amino acids, encouraged consideration of its use. Although there is a considerable literature on the stereoselectivity of the cleavage of N^2-phenylacetyl (2*S*)-amino acids by this enzyme we found no reports regarding the selectivity for N^2-phenylacetyl-(2*S*)-amino-3-hydroxy carboxylic acids using this enzyme, apart from serine which has no second chiral centre.[23] Therefore (2*S*,3*R*)-*N*-phenylacetylthreonine and its enantiomer were prepared by the conventional route.[24] The (2*S*,3*R*)-enantiomer was cleaved with immobilised *E. coli* acylase [EC 3.5.1.11] to yield (2*S*,3*R*)-threonine whilst the (2*R*,3*S*)-*N*-phenylacetylthreonine was unaffected by the enzyme, thus confirming that the acylase exhibited the same α-stereoselectivity for 3-hydroxy amino acids as it does for the 3-unsubstituted substrates. This enzymatic method was therefore deemed appropriate for the preparation of α-resolved 5-substituted 3-hydroxyornithines.

Synthesis of Protected 3-Hydroxyornithines.—A synthesis of an N^5-protected 3-hydroxyornithine derivative was required which allowed ready access to each diastereoisomeric series in a form suitable for the resolution of enantiomers. A series of papers by Shiba *et al.*[6] describe such an approach in their syntheses of capreomycidine and epicapreomycidine; however, the yields of the resolved intermediate N^5-protected 3-hydroxyornithines were rather poor. Numerous syntheses of 2-amino-3-hydroxy acids have been recently published with emphasis on diastereo- and enantio-selectivity.[25] None of these routes appear to be readily applicable to the problem in hand.

In order to distinguish between the diastereoisomers of the projected synthetic 3-hydroxyornithine derivatives a reference sample of the parent amino acids of known relative stereochemistry was required. Apart from the work of Shiba *et al.* mentioned above, Bey *et al.* reported[26] preparing 3-hydroxyornithine of unspecified relative stereochemistry from 3-phthalimidopropionaldehyde (**16**) and ethyl isocyanoacetate, but gave no experimental details. When this present work was initiated Shanzer *et al.* had recently reported[27] that *erythro*- and *threo*-2-amino-3-hydroxy acids result from lithium di-isopropylamide-induced reaction of simple aldehydes with *N*,*N*-bis(trimethylsilyl)glycine trimethylsilyl ester or *N*-benzyloxycarbonylglycine ethyl ester respectively. We were unable to isolate identifiable products when 3-(benzyloxycarbonylamino)propionaldehyde[6d] was used under the Shanzer conditions. However, compound (**16**)[28] was successfully treated with isocyanoacetamide (**17**)[29] (Scheme 4) obtained from reaction of ammonia with methyl isocyanoacetate (**18**).[30] The resulting oxazoline (**19**) possessing the thermodynamically more stable *trans* stereochemistry was isolated as a white solid. The other product of the reaction was assigned the constitution (**20**) on the basis of ^1H NMR data. The oxazoline (**19**) was hydrolysed with concentrated hydrogen bromide to give the dihydrobromide salt (**21**) of 3-hydroxyornithine as the pure *threo* diastereoisomer. Hydrolysis of compound (**20**) under the same conditions gave the salt (**21**) as a 9:1 *threo*:*erythro* (HPLC and ^1H NMR) diastereoisomeric mixture. The reaction of methyl isocyanoacetate (**18**) with aldehyde (**16**) in methanol using sodium cyanide as base[31] gave an uncharacterised intermediate which was hydrolysed,

as above, to the amino acid (**21**) as a 9:1 *threo*:*erythro* diastereoisomer ratio, in yields similar to the isocyanoacetamide route. *threo*- and *erythro*-3-Hydroxyornithines are readily assayed by derivatisation with dansyl chloride* followed by HPLC analysis. When this work was complete our *threo*- and *erythro*-3-hydroxyornithines were found to have HPLC properties identical with those of samples synthesised by an entirely different route.[32]

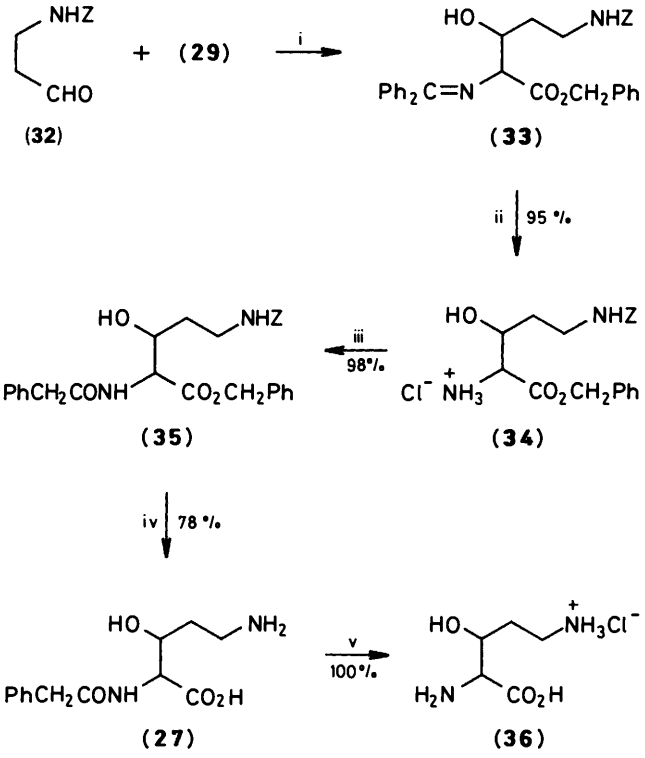
Since the routes described in Scheme 4 did not provide each diastereoisomeric series, an alternative approach was required. We reasoned that aldol condensation of 3-azidopropionaldehyde (**22**)[33] which had already proved a useful aldol synthon in our alternative synthesis of proclavaminic acid,[1,4] with a Schiff's base of a glycine ester would yield the requisite substituted carbon skeleton as a mixture of diastereoisomers amenable to functional group manipulation (Scheme 5).



Scheme 5. *Reagents and conditions:* i, $(Me_3Si)_2NLi$, THF, −70 °C. ii, 2M-HCl–ether. iii, $PhCH_2CO_2H$, $EtN{=}C{=}N[CH_2]_3NMe_2 \cdot HCl$, 1*H*-tetrazole buffer (pH 6), toluene–THF. iv, NaOH (1 mol equiv.), THF–water (2:1). v, H_2, Pd–C (5%), EtOH–water (1:1). vi, ZCl, pH 8–9.

Treatment of compound (**23**)[34] with lithium bis(trimethylsilyl)amide in tetrahydrofuran (THF) at −70 °C followed by addition of the aldehyde (**22**) gave the adduct (**24**) (Scheme 5). Acid hydrolysis unmasked the 2-amino function which was then acylated to give compound (**25**) with phenylacetic acid using 1-(3-dimethylaminopropyl)-3-ethylcarbodi-imide in 1*H*-tetrazole buffer. Column chromatography failed to separate cleanly the diastereoisomers which were present in the ratio 77:23 (HPLC). The major diastereoisomer could be isolated 95% pure by further chromatography and recrystallisation; the minor isomer could not be obtained >90% pure. The relative stereochemistry of diastereoisomers was revealed by the following transformations. Hydrolysis of the major diastereoisomer (**25**) with sodium hydroxide in aqueous THF (1:1) gave the corresponding acid (**26**) which was reduced by hydrogenation over 10% palladium–carbon catalyst to the amino acid (**27**). Cleavage of the phenylacetyl group of compound (**27**) with 48% hydrogen bromide yielded *threo*-3-hydroxyornithine, identical with the reference sample. Since the azido function is likely to react with triphenylphosphine[35] and hence would be unsuitable for masking of the 5-amino moiety during azetidinone formation, the amino group of compound (**27**) was protected by benzyloxycarbonylation to afford compound (**28**).

This route did not readily furnish both diastereoisomeric series, therefore the benzyl ester analogues were investigated, starting with compound (**29**). The aldol condensation, hydrolysis of the intermediate imine (**30**), and acylation reactions proceeded as previously to yield the ester (**31**) with an isomer ratio of 72:28 (*threo*:*erythro*). The major, *threo* isomer could be obtained pure by repeated fractional recrystallisation or alternatively the diastereoisomers could be separated by column chromatography. A more efficient route to the target intermediate (**28**) was provided by the use of 3-(benzyloxycarbonylamino)propionaldehyde (**32**) (Scheme 6).



Scheme 6. *Reagents and conditions:* i, $(Me_3Si)_2NLi$, THF, −70 °C. ii, 2M-HCl–ether. iii, $NaHCO_3$, $PhCH_2CO_2H$, $EtN{=}C{=}N[CH_2]_3NMe_2 \cdot HCl$, THF. iv, H_2, Pd–C (10%), EtOH–water (2:1). v, 5M-HCl.

Aldol reaction of aldehyde (**32**) with lithium enolate of compound (**29**) gave a high yield of the adduct (**33**) which on acid hydrolysis (2M-aq. hydrochloric acid–ether) yielded a sparingly soluble hydrochloride salt at the interface. This proved to be mainly the hydrochloride salt of the *erythro* amino ester compound (**34**). Isolation of the totally free amino ester and regeneration of the hydrochloride salt yielded the mixed diastereoisomers in the ratio 1:1 (HPLC). The hydrochloride salt of *erythro*-(**34**) was isolated in >97% diastereoisomeric purity by trituration of the mixture with ethyl acetate followed by recrystallisation. The *threo*-(**34**) was obtained from the mother liquors as a gum by further trituration. Catalytic reduction of *erythro*-(**34**) yielded *erythro*-3-hydroxyornithine hydrochloride.

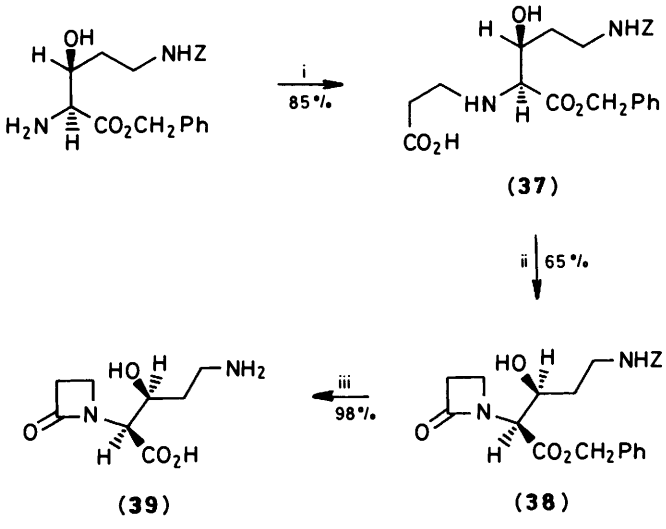
Phenylacetylation of the mixture of amino esters (**34**) gave a mixture of diastereoisomers (**35**) separable by column

* 5-Dimethylaminonaphthalene-1-sulphonyl chloride.

chromatography. The less polar diastereoisomer was shown to be *threo* by reduction to acid (**27**) and subsequent acid hydrolysis to the *threo*-amino acid (**36**). It is of interest to note that the *threo* diastereoisomers of compounds (**31**) and (**35**) were more mobile on TLC and HPLC (silica) than were the *erythro* diastereoisomers. A similar observation was made by Guanti *et al.*[25a] for *N*,*N*-dibenzyl α-amino-β-hydroxy esters. In the ^{1}H NMR spectra of the diastereoisomers of (**31**) and (**35**) the chemical shift of the 3-H was at higher field in the *threo* series than in the *erythro* series by Δδ 0.21 and 0.26, and $J_{2,3}$ was greater for *erythro* diastereoisomers (3.3 Hz) than for the *threo* diastereoisomers (2.4 and 2.1 Hz). In the ^{13}C spectra the chemical shifts of C-2 and C-3 were at higher field in the *erythro* series, by Δδ 0.60 and 1.7, for (**31**) and by Δδ 1.9 and 1.0 respectively for (**35**).

Synthesis of Proclavaminic Acid.—Since the two diastereoisomers of compounds (**34**) and (**35**) could now be satisfactorily separated in good yield the next step was to identify the relative stereochemistry of the biologically active diastereoisomer of proclavaminic acid. Both diastereoisomers of proclavaminic acid had been prepared by the direct aldol route described previously[1,4] although the relative stereochemistry of each isomer could not be assigned.

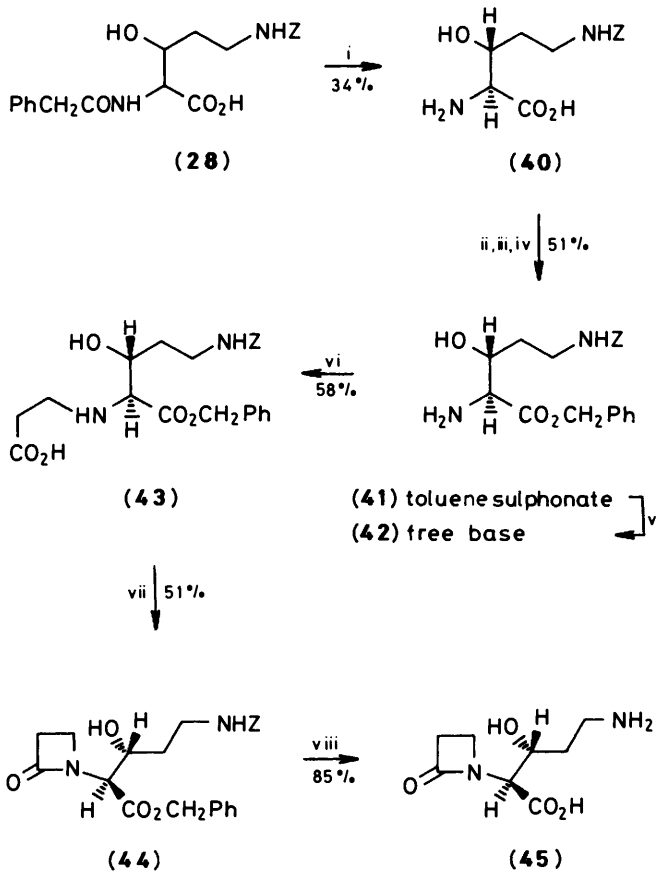
Using the methodology described above, the free base of *erythro*-(**34**) was treated with acrylic acid to yield the β-amino acid ester (**37**) (Scheme 7), which on cyclisation under the Ohno



Only one enantiomer shown

Scheme 7. *Reagents and conditions:* i, CH_2=$CHCO_2H$ (10 mol equiv.), MeCN, room temp. ii, di-2-pyridyl disulphide, Ph_3P, MeCN, reflux. iii, H_2, Pd–C (10%), EtOH–water (2:1).

conditions yielded the β-lactam (**38**)[1,4] with no detectable racemisation. Catalytic reduction of compound (**38**) yielded the free acid (**39**), the spectroscopic (^{1}H and ^{13}C) properties of which were identical with those of one of the diastereoisomers of proclavaminic acid prepared by the direct aldol route.[1,4] Neither of these materials was converted into clavaminic acid by the clavaminic acid synthase system.[36] From this result we deduced that natural proclavaminic acid possessed the *threo* relative stereochemistry, and since the 2-position was considered unlikely to be inverted during the enzymatic cyclisation to clavaminic acid (**2**) the absolute stereochemistry of proclavaminic acid is probably (2*S*,3*R*). In order to confirm this hypothesis, *threo*-(**35**) was hydrolysed to the corresponding acid



Scheme 8. *Reagents and conditions:* i, Immobilised *E. coli* acylase, pH 7.5, 37 °C. ii, KOH (1 mol equiv.), $MeCOCH_2CO_2Me$, MeOH, 70 °C. iii, $PhCH_2Br$, DMF. iv, *p*-$MeC_6H_4SO_3H$, dioxane–ethyl acetate (3:1). v, $NaHCO_3$. vi, CH_2=$CHCO_2H$ (10 mol equiv.), MeCN, room temp. vii, di-2-pyridyl disulphide, Ph_3P, MeCN, reflux. viii, H_2, Pd–C (10%), EtOH–water (7:3).

(**28**) (Scheme 8), which was treated with immobilised *E. coli* acylase [EC 3.5.1.11] to yield the (2*S*,3*R*)-amino acid (**40**). This compound was shown to be enantiomerically pure by chiral HPLC.[37] Reference mixtures of enantiomers of the known[6b] *threo*- and *erythro*-(**40**) were prepared by hydrolysis of diastereoisomers of compound (**34**). Conversion of (2*S*,3*R*)-(**40**) into the benzyl ester by MacLaren's method[38] gave the toluenesulphonate salt (**41**).

The developed route for the elaboration of the β-lactam onto the corresponding amino ester (**42**) was then applied to yield the required cyclic material (**44**). The enantiomeric purity of the product (**44**) was demonstrated to be >99% by the use of the chiral solvating reagent (*S*)-1-(9-anthryl)-2,2,2-trifluoroethanol with ^{1}H NMR analysis. Catalytic reduction of ester (**44**) produced proclavaminic acid (**45**). In cell-free reactions containing partially purified clavaminic acid synthase[36] (prepared from *Streptomyces clavuligerus*), α-ketoglutarate, and ferrous ions, the synthetic proclavaminic acid was converted into clavaminic acid (**2**) to the same degree as was natural proclavaminic acid. In parallel experiments a mixture of enantiomers of *threo*-proclavaminic acid gave exactly half the conversion into clavaminic acid as did the enantiomerically pure synthetic material and natural compound. Therefore we conclude that natural proclavaminic acid possesses the (2*S*,3*R*) absolute stereochemistry. Consequently the ring closure of the monocyclic proclavaminic acid (**45**) to the bicyclic clavaminic acid (**2**) which has the (2*S*,5*S*) stereochemistry, by clavaminic

acid synthase, proceeds with the retention of stereochemistry at the carbon bearing the carboxy function.

Experimental

M.p.s were determined on a Reichert Micro Melting Point or Gallankamp MF 370/11 apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 983 spectrophotometer. ^1H and ^{13}C NMR spectra were recorded either on a Bruker AM 250 or AM 400 spectrometer; except where otherwise stated $CDCl_3$ was used as solvent with tetramethylsilane as internal standard. In spin-echo ^{13}C NMR experiments CH_3 and CH carbon resonances are denoted by (+) and CH_2 and C resonances by (−). *J*-Values are given in Hz. DEPT in ^{13}C NMR experiments stands for distortionless enhancement by polarisation transfer. For non-equivalence measurements the ^1H NMR spectra were recorded using a solution of (*R*)- or (*S*)-1-(9-anthryl)-2,2,2-trifluoroethanol and the compound under study in the ratio 10:1 by weight in $CDCl_3$ (0.5 ml). Racemates were always checked to confirm the separation of signals due to the protons in the 2-position. Mass spectra were recorded on a VG 7070F spectrometer using electron impact (EI) or chemical ionisation (CI); for fast-atom bombardment (FAB) spectra and high-resolution spectra a VG ZAB IF double-focusing instrument was used. Optical rotations were measured on a Perkin-Elmer 141 polarimeter. HPLC was performed using columns and eluates detailed in the text. Analytical TLC was carried out on Merck pre-coated silica gel 60 F_{254} glass plates which were visualised with UV light and/or iodine vapour; TLC was carried out routinely on all reaction mixtures and final products. Column chromatography was carried out on Merck or Reidel-de-Haahn Kieselgel 60 (0.04–0.063 mm). Anhydrous magnesium sulphate was used for drying organic solutions. Acetonitrile was distilled from P_2O_5, and di-2-pyridyl disulphide and triphenylphosphine were recrystallised from hexane before use.

(S)-N^5-*Benzyloxycarbonyl*-N^2-(3-*bromopropionyl*)*ornithine Benzyl Ester* (**5**).—A solution of (*S*)-*N*^{5}-benzyloxycarbonylornithine benzyl ester (12 g, 34 mmol) in water (150 ml) and THF (225 ml) was cooled in ice and stirred while a solution of 3-bromopropionyl chloride (3.4 ml, 34 mmol) in THF (15 ml) was added dropwise during 20 min. The pH was maintained between 6.7 and 7.3 by the addition of saturated aqueous $NaHCO_3$. The THF was removed under reduced pressure, and the resulting precipitate was filtered off and dried over P_2O_5 to give (S)-N^5-*benzyloxycarbonyl*-N^2-(3-*bromopropionyl*)*ornithine benzyl ester* (**5**) as a white solid (14.4 g, 87%), m.p. 115–116 °C (from EtOAc–hexane); $[\alpha]_D^{20}$ −16.65° (*c* 2.0, EtOH) (Found: C, 56.2; H, 5.5; N, 5.85. $C_{23}H_{27}BrN_2O_5$ requires C, 56.21; H, 5.54; N, 5.7%); ν_{max}(KBr) 3 336, 3 307, 1 744, 1 681, 1 642, 1 530, 748, and 699 cm^{-1}; δ_H(250 MHz) 1.35–2.05 (4 H, m, 3- and 4-H_2), 2.67–2.92 (2 H, m, CH_2CON), 3.10–3.26 (2 H, m, 5-H_2), 3.53–3.72 (2 H, m, CH_2Br), 4.68 (1 H, dt, *J* 7.5 and 5.1, 2-H), 4.75–4.90 (1 H, s, NH), 5.09 (2 H, s, CH_2Ph), 5.17 and 5.18 (2 H, ABq, *J* 12.2, CH_2Ph), 6.39 (1 H, d, *J* 7.7, NH), and 7.34 (10 H, s, Ph).

(R)-N^5-*Benzyloxycarbonyl*-N^2-(3-*bromopropionyl*)*ornithine Benzyl Ester* (**5**).—This was prepared in 86% yield from (*R*)-*N*^{5}-benzyloxycarbonylornithine benzyl ester. The analytical data obtained were indistinguishable from those of the (*S*)-enantiomer except for the rotation: $[\alpha]_D^{20}$ +18.64° (*c* 1, EtOH).

Benzyl 5-*Benzyloxycarbonylamino*-2-(2-*oxoazetidin*-1-*yl*)*valerate* (**6**).—A solution of (*S*)-*N*^{5}-benzyloxycarbonyl-*N*^{2}-(3-bromopropionyl)ornithine benzyl ester (**5**) (3.5 g, 7.1 mmol) in DCM–acetonitrile (19:1) (240 ml) was added during 4 h to a vigorously stirred suspension of pulverised potassium hydroxide (1.04 g, 18.54 mmol) and tetrabutylammonium bromide (0.79 g, 2.5 mmol) in the same solvent mixture (240 ml). The reaction mixture was stirred for a further 2.5 h, decanted from the solid residue, evaporated to dryness, and the residue was chromatographed with ethyl acetate–hexane (1:1) as eluant to give *benzyl* 5-*benzyloxycarbonylamino*-2-(2-*oxoazetidin*-1-*yl*)*valerate* (**6**) as an oil (1.7 g, 58%), $[\alpha]_D^{20}$ −2.80° (*c* 2.0, EtOH) (Found: C, 67.5; H, 6.5%; *M*$^+$, 410.1837. $C_{23}H_{26}N_2O_5$ requires C, 67.30; H, 6.39%; *M*, 410.1842); ν_{max}(KBr) 1 737, 750, and 698 cm^{-1}; δ_H(250 MHz) 1.35–2.05 (4 H, m, 3- and 4-H_2), 2.89 (2 H, t, *J* 4, CH_2CO), 3.00–3.50 (4 H, m, 5-H_2 and CH_2N), 4.35 (1 H, dd, *J* 9.8 and 5.1, 2-H), 4.80–4.90 (1 H, s, NH), 5.06 (2 H, s, CH_2Ph), 5.12 (2 H, s, CH_2Ph), and 7.31 (10 H, s, Ph); addition of (*S*)-1-(9-anthryl)-2,2,2-trifluoroethanol caused splitting of the signals due to 2-H and both CH_2Phs, indicating the presence of a 2:1 mixture of enantiomers.

5-*Amino*-2-(2-*oxoazetidin*-1-*yl*)*valeric Acid* (**7**) *from* (S)-(**5**).—Benzyl 5-benzyloxycarbonylamino-2-(2-oxoazetidin-1-yl)valerate (**6**) (1.43 g, 3.5 mmol) [prepared by cyclisation of the (*S*) benzyl ester (**5**)] was hydrogenated with 10% palladium–carbon catalyst (1.6 g) in ethyl acetate–ethanol (7:3, 250 ml) for 15 min. The catalyst was filtered off and washed with water, and the combined filtrate was evaporated to give the *acid* (**7**) (520 mg, 80%) as a white solid, m.p. 114.5–115.5 °C (from aq. EtOH–acetone); $[\alpha]_D^{20}$ −4.8° (*c* 1.6, water) (Found: C, 49.0; H, 8.0; N, 13.9. $C_8H_{14}N_2O_3 \cdot 0.5\ H_2O$ requires C, 49.22; H, 7.75; N, 14.35%); ν_{max}(KBr) 3 422, 2 954, 2 123, 1 722, 1 641, and 1 591 cm^{-1}; δ_H(250 MHz; D_2O) 1.55–2.00 (4 H, m, 3- and 4-H_2), 2.88–2.98 (2 H, m, CH_2CO), 3.03 (2 H, t, *J* 7, 5-H_2), 3.33–3.50 (2 H, m, CH_2N), and 4.08 (1 H, dd, *J* 9.2 and 5.4, 2-H); *m/z* (FAB, thioglycerol) (Found: *M*H$^+$, 187. $C_8H_{15}N_2O_3$ requires *m/z*, 187). HPLC indicated a ratio of enantiomers *S*:*R* (63:37). The HPLC sample, prepared by derivatisation with dansyl chloride* and (*R*)-phenylalanine methyl ester, was analysed on a Zorbax C_8 column with 40% THF and 60% 0.5M-NaH_2PO_4 adjusted to pH 6 with aq. sodium hydroxide as eluant.[39]

5-*Amino*-2-(2-*oxoazetidin*-1-*yl*)*valeric Acid* (**7**) *from* (R)-(**5**).—This was prepared in a similar manner to the above experiment. The resulting 5-amino-2-(2-oxoazetidin-1-yl)valeric acid gave analytical data indistinguishable from the above except for the rotation; $[\alpha]_D^{20}$ +5.3° (*c* 1.6, water). HPLC analysis indicated a ratio of enantiomers *S*:*R* (39:61).

(2R,3S)-N-[2-(2,2,2-*Trichloroethoxycarbonyl*)*ethyl*]*threonine Benzyl Ester* (**10**).—A solution of (2*R*,3*S*)-threonine benzyl ester (**9**) (4 g, 19 mmol) in ethanol (20 ml) was stirred for 18 h with 2,2,2-trichloroethyl acrylate (**8**)[14] (3.1 g, 15 mmol) at ambient temperature. The solvent was evaporated off and the residue was chromatographed twice with chloroform and then ether–hexane (1:1) as eluant to give (2R,3S)-N-[2-(2,2,2-*trichloroethoxycarbonyl*)*ethyl*]*theonine benzyl ester* (**10**) as an oil (1.45 g, 18%); $[\alpha]_D^{20}$ +15.60° (*c* 1.6, $CHCl_3$) (Found: C, 46.3; H, 5.0; N, 3.3; Cl, 25.8. $C_{16}H_{20}Cl_3NO_5$ requires C, 46.56; H, 4.88; N, 3.39; Cl, 25.77%); ν_{max}(KBr) 1 752, 1 733, 799, 721, and 699 cm^{-1}; δ_H(90 MHz) 1.13 (3 H, d, *J* 6, Me), 1.70–3.20 (2 H, s, OH and NH, D_2O exch.), 2.45–3.20 (5 H, m, CH_2CH_2 and 3-H), 3.62 (1 H, t, *J* 7, 2-H), 4.68 (2 H, s, CH_2CCl_3), 5.14 (2 H, s, CH_2Ph), and 7.30 (5 H, s, Ph). Unchanged (2*R*,3*S*)-threonine benzyl ester (**9**) (1.2 g, 30%) was recovered.

(2R,3S)-N-(2-*Carboxyethyl*)*threonine Benzyl Ester* (**11**).—A stirred solution of (2*R*,3*S*)-*N*-[2-(2,2,2-trichloroethoxycarbon-

* 5-Dimethylaminonaphthalene-1-sulphonyl chloride.

yl)ethyl]threonine benzyl ester (**10**) (1.3 g, 3.1 mmol) was dissolved in THF (250 ml), then treated with acetic acid (50 ml, 0.87 mmol) and powdered zinc (14.1 g, 0.22 mol) in such a way that the temperature did not rise above 35 °C. The suspension was stirred at room temperature for 0.5 h. The solid material was filtered off and the filtrate was evaporated to small volume and azeotroped three times with toluene. The residual white powder was stirred with ethyl acetate (300 ml) and the undissolved solid was filtered off and washed with two aliquots of ethyl acetate. The combined filtrates were evaporated to dryness to give a white solid (1 g) containing (2*R*,3*S*)-*N*-(2-carboxyethyl)threonine benzyl ester (**11**). Comparison of the analytical data with those obtained from the synthesis using acrylic acid (see below) demonstrated the presence of (2*R*,3*S*)-*N*-(2-carboxyethyl)threonine benzyl ester and inorganic impurity.

The following procedure is illustrative of the Michael condensation reaction between acrylic acid and an α-amino ester.

(S)-N^5-*Benzyloxycarbonyl*-N^2-(2-*carboxyethyl*)*ornithine Benzyl Ester* (**15**).—A solution of N^5-benzyloxycarbonylornithine benzyl ester (2 g, 5.6 mmol) in acetonitrile (72 ml) was stirred at ambient temperature with acrylic acid (3.84 ml, 56 mmol) for 18 h. The reaction mixture was evaporated to give an oil which was triturated first with hexane (150 ml) and then twice with ether (100 ml) to give crystalline (S)-N^5-*benzyloxycarbonyl*-N^2-(2-*carboxyethyl*)*ornithine benzyl ester* (**15**) (1.34 g, 56%), m.p. 95–96.5 °C (from aq. acetone); $[\alpha]_D^{20}$ −1.63° (*c* 2.0, $CHCl_3$) (Found: C, 64.45; H, 6.6; N, 6.8. $C_{23}H_{28}N_2O_6$ requires C, 64.47; H, 6.59; N, 6.54%); ν_{max}(KBr) 1 636, 1 544, 732, and 696 cm^{-1}; δ_H(250 MHz) 1.42–1.65 (2 H, m) and 1.65–1.90 (2 H, m) (3- and 4-H_2), 2.35–2.58 (2 H, m, CH_2CO_2), 2.60–2.80 (1 H, m), 2.90–3.05 (1 H, m), and 3.06–3.24 (2 H, m) (CH_2N and 5-H_2), 3.48 (1 H, t, *J* 6.1, 2-H), 5.07 (2 H, s, CH_2Ph), 5.17 and 5.18 (2 H, ABq, *J* 12.2, CH_2Ph), 6.20–6.60 (3 H, s, CO_2H, and 2 × NH, D_2O exch.), and 7.20–7.43 (10 H, m, Ph); *m/z* (FAB, 3-nitrobenzyl alcohol) (Found: MH^+, 429. $C_{23}H_{29}N_2O_6$ requires *m/z* 429.

(2R,3S)-N-(2-*Carboxyethyl*)*threonine Benzyl Ester* (**11**).—This *solid ester* was obtained from (2*R*,3*S*)-threonine benzyl ester as described above in 78% yield; $[\alpha]_D^{20}$ +15.53° (*c* 2.0, water) (Found: C, 60.1; H, 6.8; N, 4.9. $C_{14}H_{19}NO_5$ requires C, 59.77; H, 6.81; N, 4.98%); ν_{max}(KBr) 3 256, 1 738, 1 616, 1 568, 755, and 700 cm^{-1}; δ_H(250 MHz; D_2O) 1.31 (3 H, d, *J* 6.5, Me), 2.55 (2 H, t, *J* 6.4, CH_2CO_2), 3.10–3.39 (2 H, m, CH_2N), 4.02 (1 H, d, *J* 5.8, 2-H), 4.26 (1 H, dq, *J* 6.5 and 6.2, 3-H), 5.33 and 5.35 (2 H, ABq, *J* 12.0, CH_2Ph), and 7.47 (5 H, s, Ph).

The following procedure is illustrative of the β-amino acid ring closure to form a β-lactam.

(2S)-*Benzyl* 5-*Benzyloxycarbonylamino*-2-(2-*oxoazetidin*-1-*yl*)*valerate* (**6**).—A solution of (*S*)-N^5-benzyloxycarbonyl-N^2-(2-carboxyethyl)ornithine benzyl ester (**15**) (500 mg, 1.12 mmol) in acetonitrile (60 ml) was boiled under reflux with di-2-pyridyl disulphide (258 mg, 1.17 mmol) and triphenylphosphine (307 mg, 1.17 mmol) for 6 h. The reaction mixture was evaporated to dryness and the residue was chromatographed with ethyl acetate–hexane (1:1) as eluant to give (2S)-*benzyl* 5-*benzyloxycarbonylamino*-2-(2-*oxoazetidin*-1-*yl*)*valerate* (**6**) as a clear oil (250 mg, 51%); $[\alpha]_D^{20}$ −10.95° (*c* 2.0, EtOH) (Found: C, 65.7; H, 6.2; N, 6.6. $C_{23}H_{26}N_2O_5 \cdot 0.5\ H_2O$ requires C, 65.85; H, 6.49; N, 6.68%); ν_{max}(KBr) 1 735, 751, and 698 cm^{-1}; δ_H(400 MHz) 1.50–1.65 (2 H, m), 1.70–1.85 (1 H, m), and 1.85–2.00 (1 H, m) (3- and 4-H_2), 2.90–3.00 (2 H, m, CH_2CO_2), 3.15–3.30 (3 H, m), and 3.37–3.45 (1 H, m) (CH_2N and 5-H_2), 4.40 (1 H, dd, *J* 9.9, and 5.1, 2-H), 4.77–4.84 (1 H, m, NH), 5.09 (2 H, s, CH_2Ph), 5.16 (2 H, s, CH_2Ph), and 7.27–7.42 (10 H, m, Ph); addition of (*S*)-1-(9-anthryl)-2,2,2-trifluoroethanol caused no splitting of the signals due to 2-H or the benzylic protons, thus demonstrating the presence of a single enantiomer (Found: M^+, 410.1841. $C_{23}H_{26}N_2O_5$ requires *M*, 410.1843.

(2R,3S)-*Benzyl* 3-*Hydroxy*-2-(2-*oxoazetidin*-1-*yl*)*butyrate* (**12**).—This was obtained from (2*R*,3*S*)-*N*-(2-carboxyethyl)-threonine benzyl ester (**11**) in 48% yield as an oil which crystallised after a time, m.p. 60.5–62.5 °C; $[\alpha]_D^{20}$ +4.55° (*c* 2.0, $CHCl_3$) (Found: C, 63.7; H, 6.4; N, 5.3. $C_{14}H_{17}NO_4$ requires C, 63.86; H, 6.51; N, 5.32%); ν_{max}(KBr) 1 735, 758, and 699 cm^{-1}; δ_H(250 MHz) 1.29 (3 H, d, *J* 6.5 4-H_3), 2.94–3.14 (2 H, m, CH_2CO), 3.33–3.41 (1 H, m) and 3.41–3.50 (1 H, m) (together CH_2N), 3.98 (1 H, d, *J* 7.8, OH), 4.02 (1 H, d, *J* 3.5, 2-H), 4.38–4.52 (1 H, m, 3-H), 5.22 (2 H, s, CH_2Ph), and 7.39 (5 H, s, Ph); addition of (*R*)-1-(9-anthryl)-2,2,2-trifluoroethanol caused no splitting of the signal due to the 2-H, demonstrating the presence of a single enantiomer.

(2S,3R)-*Benzyl* 3-*Hydroxy*-2-(2-*oxoazetidin*-1-*yl*)*butyrate* (**12**).—This was obtained from (2*S*,3*R*)-threonine benzyl ester (**9**) as an oil (28%) which crystallised after a time, m.p. 59–61 °C; $[\alpha]_D^{20}$ −4.60° (*c* 2.0, $CHCl_3$) (Found: C, 63.7; H, 6.4; N, 5.45%); ν_{max}(KBr) 1 730, 753, and 699 cm^{-1}; δ_H(250 MHz) 1.29 (3 H, d, *J* 6.3, 4-H_3), 2.92–3.15 (2 H, m, CH_2CO), 3.30–3.40 (1 H, m, C*H*HN), 3.40–3.50 (1 H, m, CH*H*N), 3.96 (1 H, d, *J* 8.8, OH), 4.02 (1 H, d, *J* 3.5, 2-H), 4.35–4.51 (1 H, m, 3-H), 5.22 (2 H, s, CH_2Ph), and 7.37 (5 H, s, Ph); addition of (*R*)-1-(9-anthryl)-2,2,2-trifluoroethanol caused no splitting of the signal due to the 2-H, demonstrating the presence of a single enantiomer.

(2S)-5-*Amino*-2-(2-*oxoazetidin*-1-*yl*)*valeric Acid* (**7**).—(2*S*)-Benzyl 5-benzyloxycarbonylamino-2-(2-oxoazetidin-1-yl)valerate (**6**) (130 mg, 0.31 mmol) was dissolved in a mixture of ethanol (10 ml) and water (5 ml) and hydrogenated at ambient temperature and pressure for 0.5 h with 10% palladium–carbon catalyst (130 mg). The catalyst was filtered off and washed with ethanol–water (2:1). The combined filtrates were evaporated to dryness and the residue was triturated with ether to give (2S)-5-*amino*-2-(2-*oxoazetidin*-1-*yl*)*valeric acid* (**7**) as a white solid (53 mg, 92%); $[\alpha]_D^{20}$ −21.9° (*c* 1.6, water) (Found: C, 47.3; H, 7.6; N, 13.5. $C_8H_{14}N_2O_3 \cdot H_2O$ requires C, 47.04; H, 7.90; N, 13.72%); ν_{max}(KBr) 3 450, 2 972, 1 721, 1 641, and 1 586 cm^{-1}; δ_H(250 MHz; D_2O) 1.60–2.00 (4 H, m, 3- and 4-H_2), 2.85–3.03 (2 H, m, CH_2CO), 3.03 (2 H, t, *J* 7.4, 5-H_2), 3.33–3.50 (2 H, m, CH_2N), and 4.08 (1 H, dd, *J* 9.1 and 5.4, 2-H). HPLC of a sample derivatised with dansyl chloride and (*R*)-phenylalanine methyl ester analysed on a Zorbax C_8 column, with 40% THF and 60% 0.5M-NaH_2PO_4 adjusted to pH 6 with aq. sodium hydroxide as eluant showed the presence of a single enantiomer.[39]

(2S,3R)-*Sodium* 3-*Hydroxy*-2-(2-*oxoazetidin*-1-*yl*)*butyrate* (**13**).—After neutralisation with sodium hydroxide solution the hydrogenation product of (2*S*,3*R*)-benzyl 3-hydroxy-2-(2-oxoazetidin-1-yl)butyrate was evaporated to dryness and triturated with ether to provide (2S,3R)-*sodium* 3-*hydroxy*-2-(2-*oxoazetidin*-1-*yl*)*butyrate* (**13**) in 92% yield, m.p. 169–170 °C; $[\alpha]_D^{20}$ −24.29° (*c* 2.0, water) (Found: C, 41.4; H, 5.5; N, 7.1. $C_7H_{10}NNaO_4 \cdot 0.5H_2O$ requires C, 41.12; H, 5.43; N, 6.86%); ν_{max}(KBr) 3 417, 1 716, 1 602, and 1 389 cm^{-1}; δ_H(250 MHz; D_2O) 1.21 (3 H, d, *J* 6.5, 4-H_3), 2.90–3.08 (2 H, m, CH_2CO), 3.47–3.63 (2 H, m, CH_2N), 3.99 (1 H, d, *J* 5.7, 2-H), and 4.25 (1 H, dq, *J* 6.3 and 6.3, 3-H).

(2R,3S)-*Sodium* 3-*Hydroxy*-2-(2-*oxoazetidin*-1-*yl*)*butyrate* (**13**).—This was prepared from (2*R*,3*S*)-benzyl 3-hydroxy-2-(2-oxoazetidin-1-yl)butyrate in the same fashion as its enantiomer

to give the *title compound* (**13**) as a hygroscopic white solid, m.p. 162–163 °C; $[\alpha]_D^{20}$ +22.8° (*c* 2, water) (Found: C, 42.85; H, 5.3; N, 7.2. $C_7H_{10}NNaO_4$ requires C, 43.08; H, 5.17; N, 7.18%); ν_{max}(KBr) 3 420, 1 720, 1 610, and 1 389 cm^{-1}; δ_H(250 MHz; D_2O) 1.22 (3 H, d, *J* 6.5, 4-H_3), 2.90–3.09 (2 H, m, CH_2CO), 3.46–3.64 (2 H, m, CH_2N), 4.00 (1 H, d, *J* 5.7, 2-H), and 4.26 (1 H, dq, *J* 6.4 and 6.4, 3-H).

Epimerisation of (2S,3R)-*Benzyl* 3-*Hydroxy*-2-(2-*oxoazetidin*-1-*yl*)*butyrate* (**12**).—(2*S*,3*R*)-Benzyl 3-hydroxy-2-(2-oxoazetidin-1-yl)butyrate (50 mg, 0.21 mmol) was treated in DCM (10 ml) with DBN (0.022 ml, 0.18 mmol) at room temperature for 20 h. The reaction mixture was evaporated to dryness and the residue was chromatographed with ether as eluant to yield (2*SR*,3*R*)-*benzyl* 3-*hydroxy*-2-(2-*oxoazetidin*-1-*yl*)*butyrate* (**12**) as an oil (25 mg, 52%); ν_{max}(KBr) 1 760–1 720, 753, and 699 cm^{-1}; δ_H(250 MHz) (*threo* diastereoisomer) 1.29 (3 H, d, *J* 6.5, 4-H_3), 2.90–3.10 (2 H, m, CH_2CO), 3.25–3.49 (2 H, m, CH_2N), 3.95 (1 H, d, *J* 8.8, OH, D_2O exch.), 4.02 (1 H, d, *J*, 3.6, 2-H), 4.34 (1 H, m, 3-H), 5.24 (2 H, s, CH_2Ph), and 7.37 (5 H, s, Ph); (*erythro* diastereoisomer) 1.32 (3 H, d, *J* 6.5, 4-H_3), 2.98 (2 H, t, *J* 4.2, CH_2CO), 3.25–3.49 (2 H, m, CH_2N), 4.07 (1 H, d, *J* 3.9, 2-H) superimposed upon 4.04–4.11 (1 H, m, OH, D_2O exch.), 4.25–4.40 (1 H, m, 3-H), 5.24 (2 H, s, CH_2Ph), and 7.37 (5 H, s, Ph); the diastereoisomers were in the ratio 4:1 (*theo*:*erythro*). Distillation at 200 °C and 0.3 mmHg gave a clear liquid (Found: C, 63.9; H, 6.8; N, 5.4. $C_{14}H_{17}NO_4$ requires C, 63.86; H, 6.51; N, 5.32%).

(2S,3R)-*Threonine from* (2S,3R)-N-*Phenylacetylthreonine*.—A solution of (2*S*,3*R*)-*N*-phenylacetylthreonine[24] (300 mg, 1.26 mmol) in water (30 ml) was adjusted to pH 8 with 0.1M-lithium hydroxide, immobilised *E. coli* acylase [EC 3.5.1.11] (97.3 IU) was added, and the mixture was stirred at ambient temperature for 5 h. The mixture was filtered and the filtrate was passed through a column (2.5 × 5 cm) of Dowex 50W-8X (H^+) ion-exchange resin. The column was washed with water (100 ml) and the threonine was eluted with 0.2M-ammonia (150 ml). Evaporation of the ammonia solution yielded the amino acid contaminated with phenylacetic acid (^{1}H NMR) (162.8 mg). The crude material was taken up in water (25 ml), the pH was adjusted to 3 (dil. HCl), and the mixture was extracted with ethyl acetate (3 × 25 ml). The aqueous solution was brought to pH 7 (0.1M-LiOH), absorbed onto a fresh Dowex 50W-8X (H^+) column, washed with water, and eluted as previously. Evaporation of the ammonia solution yielded (2*S*,3*R*)-threonine (118.1 mg, 83%); $[\alpha]_D^{20}$ −20.18° (*c* 2, water) {lit.,[40] $[\alpha]_D^{20}$ −28.5° (*c* 2, water)} (Found: C, 37.3; H, 7.8; N, 10.8. Calc for $C_4H_9NO_3 \cdot 5H_2O$: C, 37.50; H, 7.81; N, 10.93%). An aliquot was converted into the *N*-heptafluorobutyryl isobutyl ester[41] and assayed by GLC on a Chirasil Val III column[42] (Alltech Associates) fitted to a gas chromatograph, which showed the threonine to be 100% (2*S*,3*R*) by comparison with suitable standards. Recrystallisation of the (2*S*,*R*)-threonine from aq. ethanol gave a 37.4 mg yield; m.p. 244–246 °C (decomp.) (lit.,[43] 251–253 °C); $[\alpha]_D^{20}$ −23.4° (*c* 1, water). When (2*R*,3*S*)-*N*-phenylacetylthreonine was treated with immobilised *E. coli* acylase [EC 3.5.1.11] under identical conditions no hydrolysis to amino acid could be detected (ninhydrin) and the starting material was recovered.

trans-4,5-*Dihydro*-5-(2-*phthalimidoethyl*)*oxazole*-4-*carboxamide* (**19**).—To a stirred solution of potassium hydroxide (3.34 g, 59.6 mmol) in methanol (50 ml) was added a mixture of 3-phthalimidopropionaldehyde (**16**) (13.2 g, 65 mmol) and isocyanoacetamide (**17**) (5 g, 59.6 mmol) while the temperature was held at 0–5 °C. The stirred reaction mixture was allowed to reach 17 °C during 3.5 h after which the *dihydro-oxazole* (**19**) was recovered, as a white solid, by filtration (3.6 g, 21%), m.p. 149–151.5 °C (from MeOH) (Found: C, 58.55; H, 4.9; N, 14.5. $C_{14}H_{13}N_3O_4$ requires C, 58.53; H, 4.56; N, 14.63%); ν_{max}(KBr) 3 405, 3 348, 3 194, 2 925, 1 770, 1 709, 1 677, and 1 618 cm^{-1}; δ_H(250 MHz; [$^2H_6$]DMSO) 1.92 (2 H, m, 6-H_2), 3.70 (2 H, t, *J* 7.1, 7-H_2), 4.12 (1 H, dd, *J* 7.2 and 1.9, 4-H), 4.52 (1 H, m, 5-H), 7.19 (1 H, d, *J* 1.9, 2-H), 7.29 (2 H, d, *J* 10.9, NH_2), and 7.85 (4 H, m, ArH).

Evaporation of the mother liquors yielded a fawn solid (**20**), ν_{max}(KBr) 3 422, 1 679, 1 624, 1 585, 1 545, 1 380, and 1 124 cm^{-1}; δ_H(250 MHz; [$^2H_6$]DMSO) *inter alia* 1.75–1.85 (m, 4-H_2), 3.3 (m, 5-H_2), 4.2 (dd, *J* 7.0 and 1.8, 2-H), 4.5–4.6 (m, 3-H), 7.15–7.9 (m, ArH), and 10.99 (t, *J* 4.8, NHCO). Irradiation at δ 3.35 caused the triplet at δ 10.99 to collapse to a singlet. This material was used without further purification.

threo-3-*Hydroxyornithine Dihydrobromide* (**21**).—*trans*-4,5-Dihydro-5-(2-phthalimidoethyl)oxazole-4-carboxamide (**19**) (425 mg, 1.48 mmol) was boiled for 20 h with 47% HBr (15 ml) and anisole (7.5 ml). The cooled solution was extracted with toluene and the aqueous phase was evaporated under reduced pressure. The residue was twice redissolved in ethanol and evaporated to give a white solid, which was triturated in ethanol–ether (1:1) and the product (**21**) was recovered by filtration (443 mg, 96%), ν_{max}(KBr) 1 745 cm^{-1} (CO_2H); δ_H(250 MHz; D_2O) 1.90–2.18 (2 H, m, 4-H_2), 3.25 (2 H, m, 5-H_2), 4.05 (1 H, d, *J* 4.38, 2-H), and 4.3–4.4 (1 H, m, 3-H); δ_C(62.9 MHz; D_2O) 31.6 (C-4), 38.0 (C-5), 58.5 (C-2), 67.9 (C-3), and 170.8 (C-1); *m*/*z* (EI) 80/82 (HBr) and 130 ($M - H_2O^+$); *m*/*z* (FAB, MeOH–glycerol) (Found: *m*/*z* 149 and 297. $C_5H_{12}N_2O_3$ requires MH^+ 149 and 2*M* + H 297). The solid (**20**) from above and the mother liquors from the preparation of compound (**19**) were boiled in 47% hydrobromic acid (150 ml) with anisole (75 ml) for 16 h and worked up as above. The resulting solid was dissolved in methanol, the mixture was filtered, and the salt was precipitated with ether to yield the 3-hydroxyornithine dihydrobromide (9.4 g, 51%) as a 1:9 *erythro*:*threo* mixture.

5-*Azido*-3-*hydroxy*-N-*phenylacetylnorvaline Ethyl Ester* (**25**).—To a stirred solution of $(Me_3Si)_2NLi$ (27 ml of a 1M solution in THF, 27 mmol) at −70 °C under N_2 was added a solution of ethyl *N*-(diphenylmethylene)glycinate[34] (6.75 g, 25.25 mmol) in THF (30 ml) during 35 min. The reaction mixture was stirred at −70 °C for 15 min and was then treated with a solution of 3-azidopropionaldehyde (**22**) (6.75 g, 68.2 mmol) during 0.5 h, the temperature of the mixture being kept below −65 °C. After a further 0.5 h at −70 °C the reaction mixture was allowed to warm to room temperature, and was then poured into a mixture of ether (200 ml) and phosphate buffer pH 7 (200 ml). The organic phase was washed with water and dried. Evaporation yielded crude 5-azido-*N*-(diphenylmethylene)-3-hydroxynorvaline ethyl ester (12 g). Chromatography of an aliquot on alumina (Camag neutral Brockman Activity 1), with 10–25% ether–cyclohexane as eluant, gave ester (**24**) as an oil, ν_{max}(film) 2 100 and 1 735 cm^{-1}; δ_H(250 MHz) 1.27 and 1.28 (3 H, t, *J* 6.6, Me), 1.5–1.95 (2 H, m, 4-H_2), 3.25 (1 H, br s, OH), 3.4–3.7 (2 H, m, 5-H_2), 3.9–4.3 (4 H, m, 2- and 3-H, CH_2Me), and 7.1–7.9 (10 H, m, Ph).

The imine was hydrolysed at room temperature in a vigorously stirred mixture of ether–2M-HCl (100 ml; 1:1). The aqueous layer was separated, washed with ether, and evaporated to yield the crude hydrochloride of the amino ester as a brown oil (9 g). This material was dissolved in 1*H*-tetrazole buffer [1*H*-tetrazole (6.3 g) in water (65.5 ml); pH to 6 with 4M-NaOH] and then treated with a solution of phenylacetic acid (3.6 g, 26.5 mmol) in water (20 ml) containing NaOH (1 g, 25 mmol) followed by 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (5.4 g, 28 mmol) in a mixture of toluene (54

ml) and THF (27 ml). The reaction mixture was stirred vigorously for 1.5 h, the organic phase was separated, and the aqueous phase was extracted with ethyl acetate (2 × 100 ml). The combined organic phases were washed with water, dried, and evaporated. The residue was chromatographed (1–3% MeOH–DCM) to yield a mixture of diastereoisomers of the *title compound* (**25**) as an oil which partially crystallised on being kept (6.01 g, 74%) (77:23, *threo*:*erythro*); ν_{max}(film) 2 090, 1 730, and 1 650 cm^{-1}; δ_H(250 MHz) 1.25 and 1.26 (3 H, t, *J* 6.2, Me), 1.5–1.75 (2 H, m, 4-H_2), 3.44 (2 H, t, *J* 6.3, 5-H_2), 3.6 (2 H, s, PhCH_2), 4.05–4.3 (3 H, m, CH_2Me and 2-H), 4.62 (1 H, m, 3-H), 6.25 and 6.48 (1 H, d, *J* 7.7, *threo* NH and *J* 4.6, *erythro* NH respectively), and 7.3 (5 H, m, Ph); *m*/*z* (EI) 321 (MH^+, 6%), 221 (35), 175 (33), 103 (22), and 91 (100) (Found: MH^+, 321.1570. $C_{15}H_{21}N_4O_4$ requires *m*/*z*, 321.1563).

threo-5-*Azido*-3-*hydroxy*-N-*phenylacetylnorvaline Ethyl Ester* (**25**).—Recrystallisation of a mixture of diastereoisomers of compound (**25**) (77:23, *threo*:*erythro*) (1.85 g) from toluene–hexane yielded the title compound *threo*-(**25**) (0.55 g, 30%) as prisms, m.p. 64–66 °C (Found: C, 56.55; H, 6.3; N, 17.4. $C_{15}N_{20}N_4O_4$ requires C, 56.23; H, 6.29; N, 17.49%); ν_{max}(KBr) 3 489, 3 292, 2 101, 1 714, 1 644, 1 544, and 1 276 cm^{-1}; δ_H(250 MHz) 1.26 (3 H, t, *J* 7, Me), 1.5–1.8 (2 H, m, 4-H_2), 2.5 (1 H, br s, OH), 3.45 (2 H, t, *J* 6.5, 5-H_2), 3.64 (2 H, s, PhCH_2), 4.1–4.3 (3 H, quartet of CH_2Me at δ 4.19, *J* 7, superimposed on multiplet of 3-H), 4.62 (1 H, dd, *J* 9 and 2, 2-H), 6.23 (1 H, d, *J* 9, NH), and 7.2–7.32 (5 H, m, Ph); δ_C(100 MHz) (spin-echo) 14.1(+) 32.7(−), 43.5(−), 48.2(−), 56.5(+), 61.9(−), 69.5(+), 127.4(+), 128.9(+), 129.3(+), 134.5(−), 170.5(−), and 171.7(−).

The *erythro* diastereoisomer was not obtained pure. Its presence in mixtures with the *threo* diastereoisomer was evident from the ^1H NMR spectrum by the doubling of the $MeCH_2$ and NH peaks. Also the chemical shift of the 3-H proton of the *erythro* diastereoisomer appeared as a double triplet at δ 4.06 compared with a multiplet at δ 4.26 for the *threo* diastereoisomer. In the ^{13}C NMR spectrum the chemical shifts of all the carbon atoms except the methyl and two aromatic carbon atoms of the *erythro* diastereoisomer were distinguishable from those of the *threo* diastereoisomer; δ_C(100 MHz) 14.1(+), 32.0(−), 43.2(−), 48.0(−), 58.0(+), 62.1(−), 70.1(+), 127.5(+), 129.0(+), 129.3(+), 134.3(−), 169.7(−), and 172.3(−).

threo-5-*Azido*-3-*hydroxy*-N-*phenylacetylnorvaline* (**26**).—A solution of the *threo* ethyl ester (**25**) (750 mg, 2.3 mmol) in THF–water (2:1) (15 ml) was treated with a solution of sodium hydroxide (94 mg, 24 mmol) in water (5 ml) and stirred at room temperature for 1 h. The THF was removed under reduced pressure, and the residue was diluted with water and extracted with ethyl acetate. The aqueous layer was acidified to pH 2 with 1M-HCl, extracted with DCM, and the extract was dried. Evaporation yielded the *title compound* (**26**) as prisms (500 mg, 73%), m.p. 162–164 °C (from EtOAc) (Found: C, 53.5; H, 5.5; N, 18.9. $C_{13}H_{16}N_4O_4$ requires C, 53.41; H, 5.52; N, 19.17%); ν_{max}(KBr) 3 273, 2 097, 1 726, 1 653, 1 539, 1 266, and 713 cm^{-1}; δ_H(250 MHz; [$^2H_6$]DMSO) 1.56 (2 H, q, *J* 7, 4-H_2), 3.35 (2 H, t, *J* 6, 5-H_2), 3.53 and 3.60 (2 H, ABq, *J* 14, PhCH_2CO), 4.06 (1 H, m, 3-H), 4.31 (1 H, dd, *J* 9 and 3, 2-H), 7.25 (5 H, m, Ph), and 8.10 (1 H, d, *J* 9, NH). The title compound was also prepared from the mixture of diastereoisomers of the ethyl ester in the same manner. Trituration of the product with a small volume of DCM yielded the less soluble *threo* compound in 90% diastereoisomeric purity.

Catalytic Reduction of threo-5-*Azido*-3-*hydroxy*-N-*phenylacetylnorvaline* (**26**).—A solution of the azido acid (**26**) (0.87 g, 3 mmol) in ethanol–water (3:2) (50 ml) was hydrogenated with 5% palladium–carbon catalyst (300 mg) at room temperature until hydrogen uptake ceased. The reaction mixture was filtered through Celite, the filter bed was washed with water, and the filtrate was evaporated to yield analytically pure *threo*-3-hydroxy-N^2-phenylacetylornithine (**27**) (450 mg, 57%), m.p. 195–197 °C, identical with that prepared from *threo*-N^5-benzyloxycarbonyl-3-hydroxy-N^2-phenylacetylnorvaline benzyl ester (**35**).

3-*Hydroxyornithine Dihydrobromide* (**21**) *from* threo-(**27**).—*threo*-3-Hydroxy-N^2-phenylacetylornithine (**27**) (300 mg, 1.1 mmol) was heated under reflux in 48% hydrobromic acid (15 ml) and anisole (7 ml) for 3 h. The cooled reaction mixture was extracted with toluene (2 × 7 ml) and the aqueous phase was evaporated to dryness. The residue was re-evaporated with water (3 × 10 ml) and ethanol (2 × 10 ml). The product was triturated with ethanol–ether and the resulting solid was dried *in vacuo* to yield 3-hydroxyornithine dihydrobromide (210 mg, 66%) as a tan amorphous solid identical with the reference compound (**21**).

Preparation of threo-N^5-*Benzyloxycarbonyl*-3-*hydroxy*-N^2-*phenylacetylornithine* (**28**) *from* threo-3-*Hydroxy*-N^2-*phenylacetylornithine* (**27**).—A stirred solution of the amino acid (**27**) (400 mg, 1.5 mmol) in a mixture of water (16 ml) and methanol (5 ml) cooled in ice was treated with benzyl chloroformate (0.32 ml, 0.38 g, 2.24 mmol) in two portions during 10 min keeping the pH of the mixture between 8 and 9 with a solution of sodium hydroxide [0.6 g, 15 mmol in water (12 ml)]. A further portion of benzyl chloroformate (0.15 ml, 0.17 g, 1 mmol) was added after 30 min, and 30 min later the reaction mixture was extracted with ether (2 × 20 ml). The aqueous phase was concentrated, the pH was brought to 2 with 2M-hydrochloric acid, and then taken to dryness. The residue was taken up in methanol, the solution was filtered, and the filtrate was evaporated. The residue was then treated similarly with methanol–chloroform (1:1) to yield *threo*-N^5-benzyloxycarbonyl-3-hydroxy-N^2-phenylacetylornithine (**28**) (210 mg, 35%), identical with the material prepared by hydrolysis of the *threo* benzyl ester (**35**).

5-*Azido*-3-*hydroxy*-N-*phenylacetylnorvaline Benzyl Ester* (**31**).—This compound was prepared as a mixture of diastereoisomers (83:17) (*threo*:*erythro*) in 60% yield in the same manner as the ethyl ester (**25**). Recrystallisation of the mixture of diastereoisomers (8.4 g) from di-isopropyl ether, then toluene, yielded the threo *isomer* (**31**). (1.8 g) as prisms, m.p. 89–91 °C (Found: C, 62.8; H, 5.9; N, 14.5. $C_{20}H_{22}N_4O_4$ requires C, 62.81; H, 5.80; N, 14.65%); ν_{max}(KBr) 3 486, 3 324, 2 105, 1 711, 1 640, 1 532, 1 281, 725, and 695 cm^{-1}; δ_H(400 MHz) 1.5–1.8 (2 H, m, 4-H_2), 3.43 (2 H, dt, *J* 8 and 2, 5-H_2), 3.63 (2 H, s, PhCH_2CO), 4.26 (1 H, m, 3-H), 4.68 (1 H, dd, *J* 9 and 2.4, 2-H), 5.16 (2 H, s, OCH_2Ph), 6.24 (1 H, d, *J* 9, NH), and 7.1–7.4 (10 H, m, Ph); δ_C(100 MHz) 32.6(−), 43.6(−), 48.4(−), 56.5(+), 67.6(−), 69.8(+), and aromatics. HPLC (Spherisorb 5μ silica column, eluant 8% MeCN, 0.1% acetic acid in DCM) indicated this material contained 2% of the *erythro* isomer. Chromatography of a 65:35 mixture of diastereoisomers (*erythro*:*threo*) (1.22 g) on silica gel with ethyl acetate–hexane (1:1) as eluant yielded the *threo* isomer (150 mg), a mixture of isomers (460 mg), and the *erythro* diastereoisomer (460 mg), which on recrystallisation from ethyl acetate–hexane yielded the pure erythro *diastereoisomer* as needles (273.3 mg), m.p. 100–101 °C (Found: C, 63.05, H, 5.6; N, 14.1. $C_{20}H_{22}N_4O_4$ requires C, 62.81; H, 5.80; N, 14.65%); ν_{max}(KBr) 3 486, 3 324, 2 105, 1 711, 1 640, 1 532, 1 281, 725, and 695 cm^{-1}; δ_H(400 MHz) 1.4–1.64 (2 H, m, 4-H_2), 3.35 (2 H, m, 5-H_2), 3.6 (2 H, s, PhCH_2CO), 3.87 (1 H, br s, OH), 4.05 (1 H, dt, *J* 12 and 3, 3-H), 4.69 (1 H, dd, *J* 6.7 and 3.3, 2-H),

5.11 and 5.19 (2 H, ABq, *J* 12, OCH_2Ph), 6.52 (1 H, *J* 6.7, NH), and 7.2–7.4 (10 H, m, Ph); δ_C(100 MHz DEPT 135) 32.0(−), 43.4(−), 48.0(−), 58.2(+), 67.9(−), 70.4(+), and aromatics; *m/z* (FAB, thioglycerol) [Found: $(M + Na)^+$, 405. $C_{20}H_{22}N_4NaO_4^+$ requires *m/z*, 405].

N^5-*Benzyloxycarbonyl-3-hydroxyornithine Benzyl Ester Hydrochloride* (**34**).—To a stirred solution of $(Me_3Si)_2NLi$ (11 ml of a 1M-solution in THF, 11 mmol) at −70 °C under N_2, a solution of benzyl *N*-(diphenylmethylene)glycinate (**29**) (3.6 g, 11 mmol) in THF (15 ml) was added during 20 min. The reaction mixture was stirred at −70 °C for 20 min, then a solution of 3-(benzyloxycarbonylamino)propionaldehyde (**32**) (3 g, 14.5 mmol) in THF (15 ml) was added dropwise during 10 min and the mixture was stirred at −70 °C for 7 min. The cooling bath was removed, and the reaction mixture was allowed to warm to −20 °C then poured into a mixture of phosphate buffer at pH 7 (100 ml) and ether (150 ml). After the mixture had been thoroughly shaken the ether layer was removed, washed with water, dried, and evaporated to yield the Schiff's base (**33**) (7.35 g); *m/z* (FAB, thioglycerol) (Found: MH^+, 537. $C_{33}H_{33}N_2O_5$ requires *m/z* 537). This material was hydrolysed by being vigorously stirred with 2M-HCl (37.5 ml) and ether (40 ml) for 2 h; the mixture was filtered to remove the insoluble hydrochloride of mainly the *erythro* amino ester (**34**). The aqueous layer of the filtrate was washed with ether and combined with the solid from the filter. Ethyl acetate (50 ml) was added and the pH of the vigorously stirred mixture was adjusted to 7.75 with solid sodium hydrogen carbonate. The aqueous phase was extracted with ethyl acetate (3 × 80 ml), and the combined organic extracts were dried and evaporated to yield the title compound (**34**) (3.87 g, 95%) as an oil which partly crystallised. The diastereoisomer ratio 60:40 (*erythro*:*threo*) was determined by analytical HPLC of the dansylated[44] mixture on a Spherisorb ODS 1 column with 70% MeOH 30% 0.02M-NaH_2PO_4 (to pH 4 with H_3PO_4) as eluant. A solution of this mixture of diastereoisomers of the title compound (**34**) (5.4 g) in ethanol (50 ml) was treated with 0.25M-HCl (55 ml) and evaporated to dryness to yield the HCl salt (5.63 g, 96%) as a solid. This material was stirred with ethyl acetate (60 ml) for 1 h and filtered to yield mainly the *erythro* diastereoisomer (2.1 g) (97:3 *erythro*:*threo*). Recrystallisation from ethanol yielded the hydrochloride of the erythro *diastereoisomer* (**34**), m.p. 195–197 °C (Found: C, 58.7; N, 6.0; N, 6.9. $C_{20}H_{25}ClN_2O_5$ requires C, 58.74; H, 6.16; N, 6.85%); ν_{max}(KBr) 3 300, 1 708, 1 691, 1 272, and 695 cm^{-1}; δ_H(250 MHz; [$^2H_6$]DMSO) 1.58–1.8 (2 H, m, 4-H_2), 2.95–3.28 (2 H, m, 5-H_2), 3.99 (1 H, m, 2-H), 4.11 (1 H, d, *J* 3, 3-H), 5.0 (2 H, s, $NHCO_2CH_2Ph$), 5.20 and 5.24 (2 H, ABq, *J* 12.5, OCH_2Ph), 5.77 (1 H, d, *J* 5, N*H*CO_2CH_2), 7.25–7.5 (10 H, m, Ph), and 8.54 (3 H, s, NH_3^+); δ_C(100 MHz; [$^2H_6$]DMSO) 32.7(−), 37.1(−), 57.2(+), 65.1(−), 66.8(−), 67.0(+), 155.9(−), 167.0(−), and aromatics.

erythro-N^5-*Benzyloxycarbonyl-3-hydroxyornithine benzyl ester* was obtained as prisms by neutralisation of the hydrochloride salt, m.p. 86–87.5 °C (from $CHCl_3$–ether–hexane) (Found: C, 64.35; H, 6.3; N, 7.4. $C_{20}H_{24}N_2O_5$ requires C, 64.50; H, 6.50; N, 7.52%); ν_{max}(KBr) 3 353, 1 731, 1 694, 1 533, 1 280, 1 204, 1 171, 740, and 700 cm^{-1}; δ_H(250 MHz) 1.35–1.7 (2 H, m, 4-H_2), 2.0–2.25 (3 H, br s, NH_2 and OH, D_2O exch.), 3.23 (1 H, m, 5-H), 3.43 (1 H, m, 5-H), 3.61 (1 H, br s, 2-H), 3.88 (1 H, m, 3-H), 5.0–5.3 (5 H, m, 2 × CH_2Ph, and NH), and 7.2–7.45 (10 H, m, Ph); δ_C(100 MHz) 32.2(−), 38.0(−), 58.7(+), 66.7(−), 66.9(−), 70.5(+), 156.9(−), 173.4(−), and aromatics.

threo-N^5-*Benzyloxycarbonyl-3-hydroxyornithine benzyl ester* was obtained from the mother liquors of the HCl salts after removal of the *erythro* diastereoisomer hydrochloride, by evaporation, neutralisation, and chromatography with MeOH–DCM (1:9) as eluant to yield an oil which crystallised after a time, m.p. 88–90 °C (Found: C, 64.0; H, 6.3; N, 7.6. $C_{20}H_{24}N_2O_5$ requires C, 64.5; H, 6.42; N, 7.52%); ν_{max}(KBr) 3 327, 1 691, 1 546, 1 048, 739, and 698 cm^{-1}; δ_H(250 MHz) 1.5–1.8 (4 H, m, 4-H_2 and NH_2, integral halved on D_2O exch.), 3.2–3.6 (3 H, m, 5-H_2 and 3-H), 3.85 (1 H, m, 2-H), 5.08 (2 H, s, CH_2Ph), 5.18 (2 H, s, CH_2Ph), 5.23 (1 H, br s, $NHCO_2$), and 7.3–7.4 (10 H, m, Ph); δ_C(100 MHz) 33.7(−), 38.0(−), 58.8(+), 66.7(−), 67.0(−), 70.1(+), 157.1(−), 173.9(−), and aromatics.

erythro-3-*Hydroxyornithine Hydrochloride* (**36**).—*erythro*-N^5-Benzyloxycarbonyl-3-hydroxyornithine benzyl ester hydrochloride (**34**) (800 mg, 1.96 mmol) was hydrogenated in ethanol–water (1:1) (40 ml) in the presence of 10% palladium–carbon catalyst. Conventional work-up followed by freeze drying of the product yielded the *title compound* (**36**) as an amorphous solid (355.7 mg, 98%) (Found: C, 31.6; H, 7.1; N, 14.35. $C_5H_{13}ClN_2O_3 \cdot 0.5H_2O$ requires C, 31.33; H, 7.36; N, 14.62%); ν_{max}(KBr) 3 333, 3 000, 1 617, 1 570, 1 527, 1 136, 1 025, and 499 cm^{-1}; δ_H(250 MHz; D_2O) 1.72–2.02 (2 H, m, 4-H_2), 3.03–3.24 (2 H, m, 5-H_2), 3.83 (1 H, d, *J* 4, 2-H), and 4.15–4.27 (1 H, m, 3-H); δ_C(100 MHz; D_2O) 42.4(+), 50.9(+), 72.7(−), 81.2(−), and 184.8(+); *m/z* (FAB, thioglycerol) (Found: MH^+, 149. $C_5H_{13}N_2O_3$ requires, *m/z* 149).

N^5-*Benzyloxycarbonyl-3-hydroxy*-N^2-*phenylacetylornithine Benzyl Ester* (**35**).—A mixture of diastereoisomers of the free base of compound (**34**) (5.4 g, 14.5 mmol) and phenylacetic acid (2.14 g, 15.7 mmol) in a mixture of THF (70 ml) and DCM (70 ml) was treated with 1-(3-dimethylaminopropyl)-3-ethylcarbodi-imide hydrochloride (2.9 g, 15.1 mmol) and was stirred vigorously for 2.5 h. The solvents were removed under reduced pressure and the residue was partitioned between chloroform and water. The organic layer was washed successively with 1M-HCl, aq. $NaHCO_3$, and water, and dried to yield the *title compound* (**35**) (7.1 g, 98%) on evaporation. Analytical HPLC on a Spherisorb 5μ silica column with 45% ethyl acetate and 0.1% acetic acid in hexane as eluant indicated the ratio of diastereoisomers as 45:55 (*threo*:*erythro*). Chromatography of a similar mixture of diastereoisomers (7.6 g) over silica gel (700 g) with 10–15% acetone–chloroform as eluant yielded the less polar diastereoisomer (3.9 g) followed by a mixture of diastereoisomers (1.8 g) and the more polar diastereoisomer (1.2 g). Recrystallisation of the less polar diastereoisomer (EtOAc–hexane) yielded threo-N^5-*benzyloxycarbonyl-3-hydroxy*-N^2-*phenylacetylornithine benzyl ester* (**35**) as prisms, m.p. 135 °C (Found: C, 68.6; H, 5.9; N, 5.7. $C_{28}H_{30}N_2O_6$ requires C, 68.55; H, 6.16; N, 5.71%); ν_{max}(KBr) 3 503, 3 312, 1 710, 1 689, 1 644, 1 541, 1 272, 732, and 695 cm^{-1}; δ_H(250 MHz) 1.45–1.6 (2 H, m, 4-H_2), 3.10 (1 H, m, 5-H), 3.47 (1 H, m, 5-H), 3.61 (2 H, s, PhCH_2CO), 3.79 (1 H, d, *J* 4, OH, D_2O exch.), 4.2 (1 H, m, 3-H), 4.65 (1 H, dd, *J* 9 and 2.1, 2-H), 4.92–5.23 (5 H, m, 2 × CH_2Ph and $NHCO_2$), 6.29 (1 H, d, *J* 9, NHCO), and 7.17–7.42 (15 H, m, Ph); δ_C(100 MHz) 34.2(−), 37.3(−), 43.3(−), 56.7(+), 66.9(−), 67.2(−), 68.9(+), 157.6(−), 170.5(−), 171.7(−), and aromatics. Recrystallisation of the more polar diastereoisomer (EtOAc–hexane) yielded erythro-N^5-*benzyloxycarbonyl-3-hydroxy*-N^2-*phenylacetylornithine benzyl ester* (**35**) as plates, m.p. 114–115 °C (Found: C, 68.75; H, 6.2; N, 5.75. $C_{28}H_{30}N_2O_6$ requires C, 68.55; H, 6.16; N, 5.71%); ν_{max}(KBr) 3 384, 3 313, 1 734, 1 693, 1 552, 1 522, 1 264, 755, and 725 cm^{-1}; δ_H(250 MHz) 1.4–1.67 (2 H, m, 4-H_2), 3.12 (1 H, m, 5-H), 3.4 (1 H, m, 5-H), 3.60 (2 H, s, PhCH_2CO), 3.94 (1 H, m, 3-H), 4.12 (1 H, s, OH, D_2O exch.), 4.64 (1 H, dd, *J* 7 and 3.3, 2-H), 5.02–5.23 (5 H, m, 2 × CH_2Ph and $NHCO_2$), 6.50 (1 H, d, *J* 7, NHCO), and 7.18–7.4 (15 H, m, Ph); δ_C(100 MHz) 33.0(−), 37.8(−), 43.3(−), 57.7(+), 66.8(−), 67.5(−), 70.8(+), 157.1(−), 169.6(−), 171.9(−), and aromatics.

threo-3-*Hydroxy*-N^2-*phenylacetylornithine* (**27**).—A solution of the *threo* benzyl ester (**35**) (1.25 g, 2.5 mmol) in ethanol–water (1:1) (30 ml) was hydrogenated in the presence of 10% Pd–C catalyst (400 mg) at room temperature. After complete reduction the catalyst was filtered off through Celite and the filtrate was evaporated to yield the *title compound threo*-(**27**) (425 mg, 78%), m.p. 195–199 °C (Found: C, 57.8; H, 6.8; N, 10.4. $C_{13}H_{18}N_2O_4 \cdot 0.25\ H_2O$ required C, 57.65; H, 6.99; N, 10.34%); ν_{max}(KBr) 3 316, 1 528, 1 492, 1 402, 731, and 696 cm^{-1}; δ_H(250 MHz; D_2O) 1.6–1.85 (2 H, m, 4-H_2), 3.07 (2 H, m, 5-H_2), 3.68 (2 H, ABq, *J* 15, PhCH_2CO), 4.16 (1 H, m, 3-H), 4.20 (1 H, d, *J* 3, 2-H), and 7.28–7.44 (5 H, m, Ph); δ_C(100 MHz; D_2O) 31.6(−), 37.8(−), 43.1(−), 59.9(+), 70.5(+), 175.3(−), 176.9(−), and aromatics.

erythro-3-*Hydroxy*-N^2-*phenylacetylornithine* (**27**).—In a similar manner the *erythro* benzyl ester (800 mg, 1.6 mmol) was hydrogenated to yield the *title compound erythro*-(**27**) (430 mg, 100%), m.p. 206–207 °C (from aq. EtOH) (Found: C, 58.3; H, 6.9; N, 10.3. $C_{13}H_{18}N_2O_4$ requires C, 58.62; H, 6.81; N, 10.50%); ν_{max}(KBr) 3 3 97, 3 293, 1 625, 1 578, 724, and 692 cm^{-1}; δ_H(250 MHz; D_2O) 1.7–1.9 (2 H, m, 4-H_2), 3.04 (2 H, m, 5-H_2), 3.66 (2 H, s, PhCH_2CO), 4.04 (1 H, m, 3-H), 4.34 (1 H, d, *J* 5, 2-H), and 7.23–7.42 (5 H, m, Ph); δ_C(100 MHz; D_2O) 30.2(−), 38.0(−), 43.0(−), 60.1(+), 70.9(+), 175.1(−), 176.0(−), and aromatics.

threo-3-*Hydroxyornithine* (**36**) *from Compound* (**27**).—A mixture of the *threo-N*^{2}-phenylacetyl amino acid (**27**) (200 mg, 0.66 mmol) and 5M-HCl was boiled under reflux for 5 h, cooled, and evaporated. Water was added to the residue and was then evaporated off. The evaporation process was repeated several times. The residue was taken up in water, freeze dried, then triturated with ethanol to yield the title compound (**36**) as an amorphous solid (136.9 mg). This material was identical (250 MHz ^{1}H NMR) with the reference compound (**21**) derived from the *trans*-oxazoline (**19**). Analytical HPLC [column Spherisorb ODS1; eluant 50% MeOH, 4.5% THF, and 45.5% 0.25M-NH_4OAc (pH 7)] of the dansylated[44] material showed identical retention characteristics to the reference material (**21**) and to a sample of *threo*-3-hydroxyornithine kindly provided by Professor S. Gould, Oregon State University.

erythro-N^5-*Benzyloxycarbonyl*-N^2-(2-*carboxyethyl*)-3-*hydroxyornithine Benzyl Ester* (**37**).—This was prepared in a similar manner to compound (**15**) to give the monoester (**37**) which crystallised from the reaction mixture in 85% yield, m.p. 153–154 °C (Found: C, 62.2; H, 6.3; N, 6.3. $C_{23}H_{28}N_2O_7$ requires C, 62.15; H, 6.35; N, 6.30%); ν_{max}(KBr) 3 336, 1 740, 1 691, 1 633, 1 533, 750, and 698 cm^{-1}; δ_H(250 MHz; [$^2H_6$]-DMSO) 1.35–1.55 (1 H, m, 4-H) and 1.63–1.82 (1 H, m, 4-H), 2.31 (2 H, t, *J* 6.6, CH_2CO_2), 2.50–2.64 (1 H, m), 2.66–2.82 (1 H, m), and 2.92–3.68 (6 H, d, *J* 6.6, superimposed upon m, CH_2N, 5-H_2, 2-H, 3-H, OH and NH), 5.00 (2 H, s, CH_2Ph), 5.12 [2 H, s, CH_2Ph), superimposed upon δ 4.70–5.30 (1 H, s, NH)], 7.19 (1 H, t, *J* 5.3, NH), and 7.25–7.45 (10 H, m, Ph); *m/z* (FAB, thioglycerol) (Found: MH$^+$, 445. $C_{23}H_{29}N_2O_7$ requires *m/z*, 445).

erythro-*Benzyl* 5-*Benzyloxycarbonylamino*-3-*hydroxy*-2-(2-*oxoazetidin*-1-*yl*)*valerate* (**38**).—This was obtained in 65% yield from *erythro-N*^{5}-benzyloxycarbonyl-*N*^{2}-(2-carboxyethyl)-3-hydroxyornithine benzyl ester (**37**) in a similar manner to compound (**6**), and was identical with the material described previously.[1] HPLC showed the presence of only a single diastereoisomer (column Spherisorb 5μ silica; eluant ethyl acetate 49.85%, acetic acid 0.15%, and hexane 50%).

erythro-5-*Amino*-3-*hydroxy*-2-(2-*oxoazetidin*-1-*yl*)*valeric Acid* (**39**).—This was obtained from *erythro*-benzyl 5-benzyloxycarbonylamino-3-hydroxy-2-(2-oxoazetidin-1-yl)valerate (**38**) by catalytic reduction in 98% yield and was found to give analytical data indistinguishable from those of the *erythro* diastereoisomer prepared previously.[1,4]

threo-N^5-*Benzyloxycarbonyl*-3-*hydroxy*-N^2-*phenylacetylornithine* (**28**).—A stirred solution of the *threo* benzyl ester (**35**) (1.77 g, 3.6 mmol) in THF–water (2:1) (105 ml) was treated with aq. sodium hydroxide (0.144 g, 3.6 mmol in 8 ml) in four portions during 10 min and the mixture was stirred for 3 h at room temperature. The THF was removed under reduced pressure, and the residual aqueous solution was extracted with chloroform, then brought to pH 3 with 1M-HCl and evaporated to dryness. The residue was triturated with methanol, filtered, and the filtrate was evaporated. This process was repeated. The remaining gum was triturated with ethyl acetate (100 ml), then filtered, and the filtrate was evaporated to yield the *title compound* (**28**) (1.1 g, 76%), m.p. 157–159 °C (from EtOAc) (Found: C, 63.05; H, 5.95; N, 6.9. $C_{21}H_{24}N_2O_6$ requires C, 62.99; H, 6.04; N, 7.00%); δ_H(250 MHz) 1.4–1.6 (2 H, m, 4-H_2), 2.9–3.2 (2 H, m, 5-H_2), 2.67 (2 H, s, PhCH_2CO), 4.03 (1 H, m, 3-H), 4.11 (1 H, dd, *J* 9 and 3, 2-H), 5.00 (2 H, s, OCH_2Ph), 7.15–7.45 (11 H, m, Ph and $NHCO_2$), and 8.04 (1 H, d, *J* 9, NH); δ_C(100 MHz) 33.9(−), 37.3(−), 41.8(−), 56.5(+), 65.1(−), 68.0(+), 156.0(−), 170.5(−), 172.0(−), and aromatics.

erythro-N^5-*Benzyloxycarbonyl*-3-*hydroxy*-N^2-*phenylacetylornithine* (**28**).—In a similar manner the *erythro* ester (**35**) (0.5 g, 1 mmol) afforded the title compound as prisms (130 mg, 32%), m.p. 132–134 °C (from EtOAc) (Found: C, 62.8; H, 5.8; N, 6.9%); ν_{max}(KBr) 3 334, 1 733, 1 688, 1 609, 1 534, 1 268, and 699 cm^{-1}; δ_H ([$^2H_6$]DMSO) 1.5–1.7 (2 H, m, 4-H_2), 2.95–3.25 (2 H, m, 5-H_2), 3.50 (2 H, s, PhCH_2CO), 3.75 (1 H, m, 3-H), 4.24 (1 H, dd, *J* 8 and 6, 2-H), 5.0 (2 H, s, CO_2CH_2Ph), 7.1–7.5 (11 H, m, Ph and $NHCO_2$), and 8.24 (1 H, d, *J* 8, NHCO); δ_C(100 MHz) 33.3(−), 37.6(−), 41.9(−), 57.6(+), 65.2(−), 68.6(+), 156.1(−), 170.2(−), 171.8(−), and aromatics.

(2S,3R)-N^5-*Benzyloxycarbonyl*-3-*hydroxyornithine* (**40**).—The racemic *threo-N*^{5}-benzyloxycarbonyl-3-hydroxy-*N*^{2}-phenylacetylornithine (**28**) (1.4 g, 4.2 mmol) was suspended in water (175 ml) and the suspension was adjusted to pH 7.5 by the addition of 0.1M-NaOH. Immobilised *E. coli* acylase [EC 3.5.1.11] (262.2 IU) was then added and the mixture was stirred at 37 °C. The progress of the enzymic reaction was followed by HPLC (column Waters C-18 μ-Bondapak; eluant 25% MeCN: 75% 0.05M-NaOAc at pH 5) and the experiment was terminated after 195 min when 45% of the starting acid had been consumed. The reaction mixture was acidified to pH 2, and the immobilised enzyme was then filtered off and washed successively with ethyl acetate, chloroform, and water. The aqueous layer was brought to pH 7 with dil. ammonia and evaporated to yield a solid (1.87 g), which on trituration with methanol yielded impure title compound (270 mg). Evaporation of the methanol and trituration of the residue with chloroform yielded a further crop (550 mg). The resin-bound enzyme was resuspended in water (50 ml), the pH was adjusted to 11 with dil. ammonia, the mixture was stirred for 20 min and filtered, and the resin was washed successively with cold (100 ml) and hot water (100 ml). The combined filtrates were adjusted to pH 7 (dil. HCl) and evaporated to yield a further sample (340 mg) of impure product. The three samples of the deacylated amino acid were combined, suspended in water (3 ml), and the title compound (**40**) was filtered off (310 mg, 34%) as prisms, m.p. 214–215 °C (lit.,[6c] 212–213°C) (Found: C, 51.2; H, 6.2; N, 9.0. Calc. for $C_{13}H_{18}N_2O_5 \cdot 1.25H_2O$: C, 51.22; H, 6.72; N, 9.19%); $[\alpha]_D^{20}$ +31.58° (*c* 1, 2M-HCl); ν_{max}(KBr) 3 431, 3 309, 1 685, 1 654,

1 635, 1 549, 1 489, 1 273, 749, and 696 cm^{-1}; δ_H(250 MHz; dil. DCl in D_2O) 1.1–1.4 (2 H, m, 4-H_2), 2.7 (2 H, m, 5-H_2), 3.53 (1 H, d, *J* 3.5, 2-H), 3.71 (1 H, dt, *J* 10 and 4, 3-H), 4.53 (2 H, s, CH_2Ph), and 6.84 (5 H, m, Ph); δ_C(100 MHz; dil. DCl in D_2O, broad band-decoupled) 33.8, 37.8, 58.3, 67.3, 67.8, 159.2, 170.9, and aromatics (HPLC of the β-naphthylamide;[37] column Cyclobond I, stationary phase β-cyclodextrin; eluant 45% of 1% Et_3N in water adjusted to pH 4.0 with AcOH, 55% MeOH. This system[37] cleanly resolved all four possible stereoisomers of N^5-benzyloxycarbonyl-3-hydroxyornithine; the *threo* enantiomers were eluted before the *erythro* enantiomers.) The (2*S*,3*R*) enantiomer described above gave a single peak on this system and corresponded to the first peak of a reference mixture containing all four stereoisomers.

threo-N^5-*Benzyloxycarbonyl*-3-*hydroxyornithine*.—To a stirred solution of the *threo* amino ester (**34**) (223.3 mg, 0.6 mmol) in THF–water (1:1) (10 ml) was added a solution of lithium hydroxide (25 mg, 6 mmol) in water (2 ml) in small portions during 2 h. The THF was evaporated off and the pH of the residual solution was adjusted to pH 6.5 with 0.5M-HCl. After evaporation the residual solid was triturated with methanol to yield the title compound (112.8 mg, 67%) as prisms, m.p. 214–218 °C (lit.,[6c] 212–213 °C) (Found: C, 53.8; H, 6.6; N, 6.9. Calc. for $C_{13}H_{18}N_2O_5 \cdot 1.5H_2O$: C, 53.60; H, 6.57; N, 6.92%); ν_{max}(KBr) 3 432, 3 331, 1 689, 1 657, 1 636, 1 550, 1 490, 1 275, 749, and 696 cm^{-1}; δ_H(250 MHz; dil. DCl in D_2O) 1.48–1.7 (2 H, m, 4-H_2), 3.00–3.15 (2 H, m, 5-H_2), 3.87 (1 H, d, *J* 3.5, 2-H), 4.06 (1 H, dt, *J* 10 and 6, 3-H), 4.88 (2 H, s, CH_2Ph), 4.91 (1 H, s, $NHCO_2$), and 7.10–7.25 (5 H, m, Ph); δ_C(100 MHz; dil. DCl in D_2O, DEPT 135) 33.6(−), 37.6(−), 58.2(+), 67.3(+), 67.7(−), and aromatics.

erythro-N^5-*Benzyloxycarbonyl*-3-*hydroxyornithine*.—In a similar manner the *erythro* amino ester hydrochloride (500 mg, 1.22 mmol) yielded the title compound (301.8 mg, 87%), m.p. 226–228 °C (from aq. EtOH) (lit.,[6b] 225–227 °C; lit.,[6d] 248–248.5 °C) (Found: C, 55.4; H, 6.35; N, 9.7. Calc. for $C_{13}H_{18}N_2O_5$ requires C, 55.30; H, 6.43; N, 9.92%); ν_{max}(KBr) 3 453, 3 301, 1 689, 1 584, 1 549, 1 326, 1 291, and 1 278 cm^{-1}; δ_H(400 MHz; dil. DCl in D_2O) 1.36–1.45 (2 H, m, 4-H_2), 2.76–2.95 (2 H, m, 5-H_2), 3.79 (2 H, m, 2- and 3-H), 4.16 (2 H, s, PhCH_2), 4.97 (1 H, br s, $NHCO_2$), and 6.9–7.07 (5 H, m, Ph); δ_C(100 MHz; dil. DCl in D_2O, DEPT 135) 32.6(−), 37.9(−), 58.0(+), 67.6(−), 67.8(+), and aromatics.

(2S,3R)-N^5-*Benzyloxycarbonyl*-3-*hydroxyornithine Benzyl Ester Toluene*-p-*sulphonate* (**41**).—A mixture of (2*S*,3*R*)-N^5-benzyloxycarbonyl-3-hydroxyornithine (**40**) (400 mg, 1.42 mmol), methyl acetoacetate (162 mg, 1.39 mmol), and potassium hydroxide (79 mg, 1.42 mmol) in methanol (2.5 ml) was stirred at room temperature for 4 h, then more methanol (3 ml) added and the mixture was stirred overnight. Further methyl acetoacetate (50 mg, 0.43 mmol) was added and mixture was warmed to 70 °C for 30 min, then cooled. The solvent was evaporated off and the residue was dried over P_2O_5 for 48 h to yield the crystalline Dane salt (0.52 g). This material was dissolved in dry dimethylformamide (DMF) (4 ml), benzyl bromide (0.26 g, 0.18 ml, 1.5 mmol) was added, and the mixture was stirred at room temperature for 4 days. The reaction mixture was diluted with ethyl acetate and washed successively with aq. 1M-$NaHCO_3$ and water, and dried. The residue remaining after evaporation of the solvents was dissolved in a mixture of dioxane (3 ml) and ethyl acetate (1 ml), then toluene-*p*-sulphonic (tosic) acid monohydrate (285 mg, 1.5 mmol) was added and the mixture was stirred overnight. The solvents were removed under reduced pressure and the residue was triturated with ether to yield the *title compound* (**41**) (400 mg, 51%) as off-white prisms, m.p. 123–125 °C (Found: C, 58.4; H, 5.7; N, 5.05; S, 5.6. $C_{27}H_{32}N_2O_8S \cdot 0.5H_2O$ requires C, 58.57; H, 6.0; N, 5.06; S, 5.79%); $[\alpha]_D^{20}$ +33.0° (*c* 1.0, water); ν_{max}(KBr) 3 421, 1 747, 1 696, 1 521, 1 255, 1 126, 1 036, 1 011, and 679 cm^{-1}; δ_H(400 MHz; [$^2H_6$]DMSO) 1.57–1.7 (2 H, m, 4-H_2), 2.28 (3 H, s, Ar*Me*), 3.0–3.2 (2 H, m, 5-H_2), 4.02–4.2 (2 H, m, 2- and 3-H), 5.0 (2 H, s, CH_2Ph), 5.18 and 5.28 (2 H, ABq, *J* 13, CH_2Ph), 7.11 (2 H, d, *J* 8, ArH), 7.2–7.48 (10 H, m, Ph), 7.48 (2 H, d, *J* 8, ArH), and 8.27 (3 H, br s, NH_3^+); δ_C(400 Mz; [$^2H_6$]DMSO) 20.7(+), 33.5(−), 37.1(−), 58.9(+), 65.2(−), 66.4(+), 67.2(−), 168.1(−), and aromatics.

(2S,3R)-N^5-*Benzyloxycarbonyl*-N^2-(2-*carboxyethyl*)-3-*hydroxyornithine Benzyl Ester* (**43**).—The tosate salt (**41**) (420 mg, 0.76 mmol) was partitioned between ethyl acetate (60 ml) and water (10 ml) and the pH of the aqueous phase was adjusted to 7.7 with 0.2M-NaOH. The ethyl acetate layer was removed, the aqueous phase was re-extracted with ethyl acetate (60 ml), and the combined organic extracts were dried. Evaporation yielded the amino ester (287 mg, 101%), identical with the racemic compound by ^1H NMR and IR spectroscopy.

The amino ester (279 mg, 0.74 mmol), acrylic acid (0.54 g, 0.52 ml, 7.5 mmol), and acetonitrile (12 ml) were stirred at room temperature overnight, then the solvent was removed under reduced pressure. The residue was triturated with hexane and then ether to yield the title compound (**43**) (200 mg, 58%) as a solid, m.p. 83–84 °C (Found: C, 61.8; H, 6.3; N, 6.2. $C_{23}H_{28}N_2O_7$ requires C, 62.15; H, 6.35; N, 6.30%); ν_{max}(KBr) 3 312, 1 728, 1 690, 1 543, 1 260, 752, and 697 cm^{-1}; δ_H(250 MHz; [$^2H_6$]DMSO) 1.5–1.7 (2 H, m, 4-H_2), 2.33 (2 H, t, *J* 6, 5-H_2), 2.56 (1 H, t, *J* 7, C*H*HCO_2), 2.8–3.2 (3 H, m, CH*H*CO_2 and CH_2N), 3.24 (1 H, d, *J* 4, 2-H), 3.25–3.6 (2 H, br s, NH, D_2O exch.), 3.78 (1 H, m, 3-H), 5.0 (2 H, s, CH_2Ph), 5.12 and 5.5 (2 H, ABq, *J* 13, CH_2Ph), 7.23 (1 H, t, *J* 5, $NHCO_2$, D_2O exch.), and 7.28 (10 H, m, Ph); *m/z* (FAB, thioglycerol) (Found: *M*H^+, 445. $C_{23}H_{29}N_2O_7$ requires *m/z*, 445).

(2S,3R)-*Benzyl* N^5-*Benzyloxycarbonylamino*-3-*hydroxy*-2-(2-*oxoazetidin*-1-*yl*)*valerate* (**44**).—The acid ester (**43**) (190 mg, 0.43 mmol), di-2-pyridyl disulphide (112 mg, 0.51 mmol), triphenylphosphine (133 mg, 0.51 mmol), and acetonitrile (50 ml) were boiled under reflux for 7.5 h. Evaporation and chromatography (5–7.5% acetone in $CHCl_3$ as eluant) afforded the title compound (**44**) (93.8 mg, 51%) as a thick oil, $[\alpha]_D^{20}$ +20.2° (*c* 1, $CHCl_3$); ν_{max}(KBr) 3 369, 1 725, and 1 527 cm^{-1}; δ_H(250 Mz) 1.68 (2 H, m, 4-H_2), 3.01 (2 H, m, CH_2CO), 3.2–3.57 (4 H, m, CH_2N and 5-H_2), 4.14 (1 H, d, *J* 3, OH), 4.29 (1 H, m, 3-H), 4.47 (1 H, d, *J* 8, 2-H), 5.08 (2 H, s, PhCH_2), 5.10 (1 H, s, NH), 5.14 (2 H, s, PhCH_2), and 7.36 (10 H, s, Ph); addition of (*S*)-1-(9-anthryl)-2,2,2-trifluoroethanol caused no splitting of the signal due to the 2-H, demonstrating the presence of a single enantiomer; δ_C(100 MHz; broad band-decoupled) 34.5, 36.3, 37.8, 40.4, 62.2, 66.9, 67.5, 68.9, 157.0, 168.5, 169.2, and aromatics.

(2S,3R)-5-*Amino*-3-*hydroxy*-2-(2-*oxoazetidin*-1-*yl*)*valeric Acid*. (*Proclavaminic Acid*) (**45**).—The protected azetidinone (**44**) (72 mg, 0.17 mmol) in ethanol–water (7:3) (20 ml) containing 10% palladium–carbon catalyst (40 mg) was hydrogenated at room temperature until uptake ceased. Conventional work-up yielded a gum (32.5 mg), which was triturated with ether; the residue was dissolved in water (3 ml) and freeze dried to yield proclavaminic acid (**45**) as a powder (29 mg, 85%), identical with the material prepared previously.[1,4]

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